Genetic Analysis of Taste in *Homo Sapiens*

by

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A Thesis

submitted to the Department of Biological Sciences

in partial fulfillment of the requirements

for the degree of

Master of Science

December, 2012

Brock University

St. Catharines, Ontario

ABSTRACT

There are many known taste receptors specific to each taste attribute. This thesis examines the relationship between single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) in known taste and taste pathway receptors TAS2R38, Gustin, and TRPM5 and for PROP (6-n-propylthiouracil) taster status (PTS), thermal taster status (TTS), and orosensory sensation intensity ratings. PTS is a proxy for general taste responsiveness, and the ability to taste PROP classifies individuals into three phenotypes: super (PST), medium (PMT), and non-tasters (PNT). Another taste phenotype, also serving as a proxy for general taste responsiveness, is TTS, classifying individuals as thermal tasters (TTs) or thermal non-tasters (TnTs). DNA extractions from buccal cells obtained from 60 individuals were performed and analysis of TAS2R38, Gustin, and TRPM5 variations were conducted through Polymerase Chain Reaction (PCR), sequencing for SNPs, and upQMPSF for CNV analysis of TRPM5. Among the SNPs and CNVs studied, only TAS2R38 was found to be significantly associated with PTS and intensity ratings for sweet, bitter, and sour taste as well as astringency. However, not all PROP phenotypic differences can be explained by the variations at these three SNP sites in TAS2R38, suggesting the involvement of additional genes. No association was found between TTS and TAS2R38 or Gustin, confirming that PTS and TTS are not genetically associated. The examined TRPM5 SNPs and CNVs did not correlate with TTS. Therefore, further research is necessary into other factors contributing to PTS and TTS.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Dr. Ping Liang, for his guidance, encouragement and hours of help troubleshooting throughout the past two years. I would also like to thank Dr. Gary Pickering for all of his help with data collection, the sensory evaluation portion of this thesis, input of data from his laboratory, use of his laboratory facilities and service as a member of my supervisory committee. Also, I would like to thank Dr. Glenn Tattersall for his valuable input, suggestions and service on my supervisory committee.

I would like to extend my greatest gratitude to Lynda Van Zuiden for her assistance in my sensory evaluation prep and in searching for data from previous studies. Great thanks also go to Dr. Martha Bajec, for the mouthwash samples collected and data for PROP and thermal taster status of many of the subjects used in this thesis.

I would like to extend thanks to my many lab mates over the past two years, especially Scott Golem, Patrick Viel, and Calvin Sjaarda, all of whom made the hours spent in the lab fly by and were always willing to have questions bounced off them. The lab would not have been the same without the three of you. I am sincerely grateful for all of the support and constant encouragement of my family and friends, especially my amazing husband, Richard, who was always willing to listen my concerns about the hurdles of research and countless explanations of molecular biology, although likely having no idea what I was talking about. Lastly, I would like to thank my parents, Tom and Sharon Holder, for their constant support and encouragement throughout my education.

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LIST OF ACRONYMS AND ABBREVIATIONS

Acronym/Abbreviation	Expansion
AA	amino acid
AAI	alanine-alanine-isoleucine
ASICs	acid sensing ion channels
ATP	adenosine-5'-triphosphate
AVI	alanine-valine-isoleucine
b	bitter
BMI	body mass index
bp	base pairs
CA6	carbonic anhydrase VI
CNV	copy number variation
DAG	diacyl glycerol
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ENaC	amiloride-sensitive epithelial sodium ion channel
gLMS	general labeled magnitude scale
GMP	guanosine monophosphate
gVAS	generalized visual analog scale
HCNs	hyperpolarization activated channels
IMP	inosine monophosphate
IP3	inositol-1,4,5-triphosphate
ISO	international standardization organization
kb	kilobase
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1
KO	knock-out
MAF	minor allele frequency
mRNA	messenger ribonucleic acid
nPROP	normalized PROP rating
nPTS	normalized PROP taster status
NTPs	nucleoside triphophates
PAV	proline-alanine-valine
PCR	polymerase chain reaction
PKD1L3	polycystic kidney disease 2-like 1 protein
PKD2L1	polycystic kidney disease 1-like 3 protein
PLC	protein lipase C
PMT	PROP medium taster

Acronym/Abbreviation	Expansion
PNT	PROP non-taster
PRB1	basic salivary proline-rich protein 1
PROP	6-n-propylthiouracil
PST	PROP super taster
PTC	phenylthiocarbamide
PTS	PROP taster status
PVI	proline-valine-isoleucine
R1	reaction one
R2	reaction two
rpm	revolutions per minute
sa	salty
SNP	single nucleotide polymorphism
so	sour
sw	sweet
TAS1R	taster receptor type 1
TAS1R1	taste receptor type 1, member 1
TAS1R2	taste receptor type 1, member 2
TAS1R3	taste receptor type 1, member 3
TAS2R	taste receptor type 2
TAS2R3	taster receptor type 2, member 3
TAS2R4	taster receptor type 2, member 4
TAS2R5	taster receptor type 2, member 5
TAS2R13	taste receptor type 2, member 13
TAS2R16	taster receptor type 2, member 16
TAS2R19	taste receptor type 2, member 19
TAS2R38	taster receptor type 2, member 38
Tm	melting temperature
TnTs	thermal non-taster
TRPM4	transient receptor potential melastatin 4 channel
TRPM5	transient receptor potential melastatin 5 channel
TRPM8	transient receptor potential melastatin 8 channel
TSPAN32	tetraspanin 32
TTS	thermal taster status
TTs	thermal taster
upQMPSF	quantitative multiplex PCR of short fluorescent fragments
UV	ultra-violet

SECTION I: INTRODUCTION

Humans have five basic tastes: sweet, sour, bitter, umami (savory), and salty. These tastes are detected through different cellular signal pathways in the oral cavity. The interaction of taste compounds with the receptors in taste buds results in the electrical excitation of the taste receptor cells, leading to the excitation of neurons that transmit the signal to the brain. This results in an increased appetite for positive tastes, such as sweet or salty, or an adverse response to negative tastes such as bitter or sour. Many of the taste receptors are also found throughout the digestive tract and other parts of the body, and they can result in physiological responses (Roudnitzky et al., 2011; Rozengurt & Sternini, 2007; Shah et al., 2009; Tizzano et al., 2010). There are three types of taste cells: Types I, II and III. Chaudhari and Roper (2010) explained the hypothesis that Type I and III cells are responsible for salty and sour taste detection/signaling respectively, and Type II cells are responsible for bitter, sweet, and umami taste detection through G-protein coupled receptors. Each taste modality has its own mechanism of perception and signaling as outlined in Figure 1.

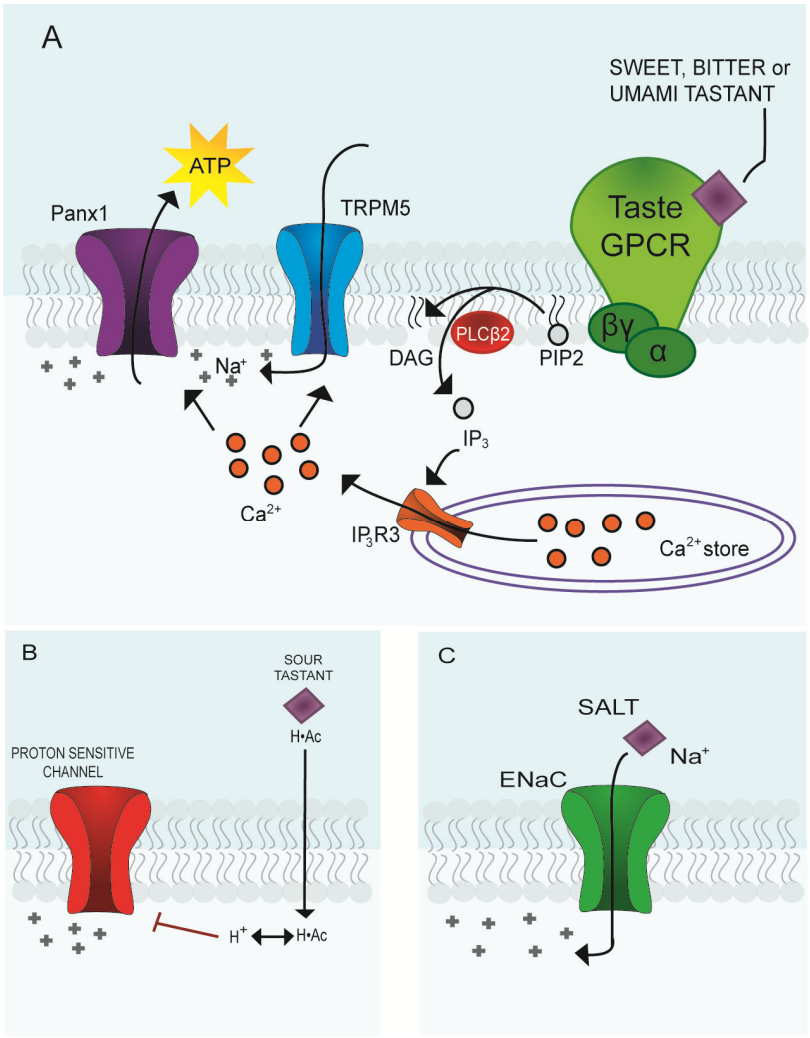

Figure 1: Molecular mechanisms for taste transduction in taste cells. (A) Type II taste cells for sweet, bitter, and umami ligands bind to G-protein coupled receptors (TAS1R or TAS2R), resulting in activation of the phosphoinositide pathway and leading to an increase in calcium in the cytoplasm depolarizing the cell through TRPM5. This depolarization leads to the opening of the Panx1 channels, resulting in ATP release. (B) Type III cells allow for organic acids to travel through the cell membrane and result in acidification of the cytoplasm, leading to the proposed inhibition of a proton-sensitive potassium channel, resulting in cell depolarization. Cell depolarization would cause an increase in cytoplasmic calcium ions, resulting in exocytosis of synaptic vesicles. (C) Detection of salty taste is the result of sodium ions permeating through ion channels such as ENaC resulting in cell depolarization (adapted from Chaudhari & Roper, 2010).

Sweet taste

As outlined in Figure 1, sweet taste is detected through the heterodimer formed by TAS1R2 (taste receptor Type 1 Member 2) and TAS1R3 (taste receptor Type 1 Member 3). This heterodimer responds to sugars, synthetic sweeteners, and sweet-tasting proteins (Jiang et al., 2004; Nelson et al., 2001; Xu et al., 2004). Damak et al. (2003) have also suggested that other alternative receptors may be involved in sweet taste transduction because mice lacking the TAS1R3 gene are still sensitive to the sweetness elicited by some sugars, although these receptors and their mechanisms have yet to be identified.

Umami taste

Similar to sweet taste transduction, umami taste is detected through a heterodimer G-protein-coupled receptor of TAS1R1 (taste receptor Type 1 Member 1) and TAS1R3. Umami taste is typically the response to l-glutamate, guanosine monophosphate/inosine monophosphate (GMP/IMP), the hydrolysis of proteins, or nucleoside triphosphates (NTPs) (Li et al., 2002; Nelson et al., 2002). As with sweet taste transduction, the TAS1R3 knockout mice still perceived umami taste, indicating the role of other receptors for umami taste, which are not yet known (Damak et al., 2003; Maruyama et al., 2006; Yasumatsu et al., 2009). It has been proposed that other G-protein-coupled receptors and their isoforms found in taste cells may be responsible for umami taste transduction (Chaudhari et al., 2000; Li et al., 2002; Nelson et al., 2002; San Gabriel et al., 2009).

Sour taste

Sour taste is associated with acidic foods and drinks. It can also be associated with food aversion and avoidance in animals particularly, as acidic taste is associated with spoiled and unripe foods (Lindemann, 1996). Sour taste can be elicited by a few mechanisms such as extracellular pH and intracellular acidification (Chang et al., 2010; Huang, Maruyama et al., 2008; Lyall et al., 2006; Taylor, 1928). Extracellular hydrochloric acid solutions of greater than 1mM or a pH of 3 are known to signal sour taste transduction (Chang et al., 2010; Taylor, 1928). In addition to extracellular pH, intracellular acidification also signals sour taste transduction through the entrance of hydrogen ions into the cell via cell membrane receptors. There are several candidate receptors for hydrogen ion transport into the cell. These receptors include acid sensing ion channels (ASICs), hyperpolarization-activated channels (HCNs), two-pore-domain K+ channels, and the PKD2L1/PKD1L3 (polycystic kidney disease 2-like 1 protein/1-like 3 protein) heterodimer (Gilbertson et al., 1993; Ishimaru et al., 2006; Kataoka et al., 2008; Lin et al., 2002; Liu & Simon, 2001; Richter et al., 2004; Stevens et al., 2001; Ugawa et al., 1998). Currently, only the PKD2L1/PKD1L3 heterodimer has been found to be expressed in sour-responsive taste cells (Chang et al., 2010). Further research is needed because no loss-of-function studies have resulted in loss of sour taste perception in animals (Horio et al., 2011), although by removing PKD2L1 through genetic manipulation, a substantial decrease in sour taste perception has been observed (Huang et al., 2008). In addition to the PKD2L1 elimination studies, Huque et al. (2009) found that individuals with sour ageusia (taste blindness) do not express PKD2L1 and PKD1L3

mRNA in regions of the tongue, suggesting that these genes are necessary for sour taste perception (Horio et al., 2011).

Salty taste

Salty taste is mainly the gustatory perception of sodium ions (Lindemann, 1997). These sodium ions travel through cell membrane receptors that are salt-specific, such as the amiloride-sensitive epithelial sodium ion channel of the ENaC family in rodents (Canessa et al., 1994). ENaC is composed of three homologous subunits that create a pathway for sodium ions into the cell if the extracellular concentration (within the oral cavity) is high enough (Kretz et al., 1999; Lin et al., 1999). This results in synaptic signaling leading to the neurological recognition of salty taste (Avenet & Lindemann, 1991). Very little is known about human salty taste transduction; however, ENaC is believed to be involved but not necessarily as the only ion channel (Lindemann, 2001; Smith & Ossebaard, 1995).

Bitter taste

Bitter taste innately triggers rejection behaviors (Beauchamp & Mennella, 2009; Chandrashekar et al., 2006; Steiner, 1973). This response is believed to be a protective behavior to prevent the ingestion of potentially harmful compounds, particularly those commonly found in scavenging mammals (Drewnowski & Gomez-Carneros, 2000; Lindemann, 1996). Many of the bitter compounds consumed by animals and humans are plant- and animal-derived. These compounds often result from food spoilage, aging, processing or from Malliard and fermentation reactions, such as in alcoholic beverages

(Belitz & Wieser, 1985; Drewnowski & Gomez-Carneros, 2000; DuBois et al., 2008; Hofmann, 2005; Kingsbury, 1964; Murata & Sata, 2000). Bitter compounds are known to be structurally diverse and as a result there are 25 known bitter taste receptors in the TAS2R (Type 2 taste receptors) family in humans. Some of the known bitter tastants include hydroxy fatty acids, fatty acids, peptides, amino acids, amines, amides, azacycloalkanes, N-heterocyclic compounds, ureas, thioureas, carbamides, esters, lactones, carbonyl compounds, phenols, crown ethers, terpenoids, ecoiridoids, alkaloids, glycosides, flavonoids, steroids, halogenated or acetylated sugars, and metal ions (Belitz & Wieser, 1985; DuBois et al., 2008; Meyerhof et al., 2010).

The TAS2R proteins are a group of 7-transmembrane receptors with ligand binding domains on the extracellular regions of the receptors. Interestingly, the TAS2R receptors are coded for by intronless genes on chromosomes 7 and 12 as gene clusters, plus a single TAS2R gene on chromosome 5 (Meyerhof et al., 2011). TAS2R receptors are also highly conserved across primates and other mammalian species such as *Mus musculus,* indicating their importance in survival and toxin avoidance (Conte et al., 2003). Much work has recently been performed in the identification of the ligands for each of the 25 TAS2R receptors in humans. Some of the receptors were found to respond to only two to four ligands, whereas other receptors were activated by many ligands (Meyerhof et al., 2010). Meyerhof et al. (2010) found that 63 of the tested ligands were detected by only one to three receptors, whereas 19 of the tested ligands were detected by up to 15 of the TAS2R receptors. The result of multiple bitter receptors, along with the genetic polymorphisms within them, allows for the detection of a large range of

bitterants, including those that are toxic and necessary to avoid for survival (Chaudhari & Roper, 2010).

In addition to their role in taste transduction, TAS2R receptors have been suggested to have a physiological role in other pathways. TAS2Rs have been found to be expressed in cells within the digestive tract, nose, and bronchi, along with the expression of other components of the bitter transduction pathway. A few studies have shown that these receptors are capable of detecting agonists and signaling through the TAS2R pathway (Roudnitzky et al., 2011; Rozengurt & Sternini, 2007; Shah et al., 2009; Tizzano et al., 2010). The most studied ligands for bitter taste are phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), although recent research has shifted to the use of PROP for perception studies because PTC was found to be carcinogenic (Lawless, 1980; Wheatcroft & Thornburn, 1972).

PROP taster status

6-n-propylthiouracil (PROP) is a bitterant used as a proxy for taste responsiveness. PROP taster status (PTS) is a classification method for responsiveness to PROP resulting in three classifications based on intensity ratings. A person's ability to taste PROP can be classified as super tasting (PST), medium tasting (PMT) and non-tasting (PNT) (Duffy et al., 2004; Pickering et al., 2006). PSTs perceive PROP at greater intensity than PMTs and PNTs; PMTs perceive PROP at moderate intensity; PNTs perceive PROP as absent or at low intensity (Duffy et al., 2004).

Genes associated with PROP taster status

As outlined previously, there are 25 TAS2R receptors in humans that are responsible for bitter taste transduction. Of these 25 receptors, TAS2R38 (taste receptor Type 2 Member 38) is known to be associated with PROP taster status (Duffy et al., 2004). The TAS2R38 gene is located on chromosome 7q36 and encodes for a 7-transmembrane domain heterotrimeric guanine nucleotide-binding protein (G-protein) coupled receptor (Lindemann, 2001). The TAS2R38 receptor signals through a signal transduction pathway as follows: the transduction pathway starts with the binding of the bitter compound (PROP) to the 7-transmembrane receptor that is coupled to a G-protein; the binding results in the separation of the G-protein subunits (Gɣ13 and Gβ3) and leads to the activation of phospholipase Cβ2, followed by the formation of inositol-1,4,5-triphosphate (IP_3); IP_3 results in the release of calcium into the cytosol and leads to a membrane potential change (Lindemann, 2001). The transduction pathway following the membrane potential change is not yet known.

There are three common TAS2R38 genotypes associated with PROP taster status. These genotypes are the result of single nucleotide polymorphisms (SNPs) at three sites within the gene. These SNPs lead to amino acid substitutions from proline-alanine-valine (PAV) to alanine-valine-isoleucine (AVI) of amino acids 49, 262 and 296 respectively (Kim et al., 2003) (see Figure 2). The PAV genotype is the result of the nucleotides, cytosine, cytosine, and guanine at the three sites respectively, whereas the AVI genotype is the result of the nucleotides, guanine, thymidine, and adenine at the three sites respectively. The heterozygous genotype of PAV/AVI is also common.

The PAV/PAV, PAV/AVI, and AVI/AVI genotypes are associated with PROP super tasters, medium tasters, and non-tasters, respectively (Duffy et al., 2004). The PAV TAS2R38 genotype recognizes compounds containing the N–C=S moiety, whereas the AVI genotype does not (Duffy & Bartoshuk, 2000). The N–C=S moiety is present in the two widely studied bitter compounds PTC and PROP (Duffy & Bartoshuk, 2000), and it is responsible for the binding of the bitterants to TAS2R38 and the initiation of taste transduction. The SNPs for the three sites in TAS2R38 and how they affect the ability to taste PROP are outlined in Figure 2. There are also other haplotypes observed such as AAV, AAI, and PVI (Duffy et al., 2004), although these haplotypes most often occur as heterozygotes with either the PAV or AVI genotype and rarely as homozygotes (Hayes et al., 2008). The TAS2R38 genotypes are inherited through incomplete dominance (Reed, 2004) in which the heterozygote for a gene exhibits a phenotype between the dominant and recessive traits. In this case, PROP medium tasters are this phenotype.

1. Cytosine (C) → Proline (**P**)
2. Cytosine (C) → Alanine (**A**) → N-–C=S binding → PROP taster
3. Guanine (G) → Valine (**V**)

1. Guanine (G) → Alanine (**A**)
2. Thymidine (T) → Valine (**V**) → ***no*** N–C=S binding → PROP non-taster
3. Adenine (A) → Isoleucine (**I**)

Figure 2: Nucleotide changes at each of the three known SNP sites within TAS2R38. The resulting amino acid substitutions and the outcome on the TAS2R38 bitter taste receptor and PROP taster status are stated. Site 1 is the first base in the codon for the 49th amino acid; site 2 is the second base in the codon for 262nd amino acid; and site 3 is first base in the codon for the 296th amino acid.

In addition to TAS2R38's contribution to PROP taster status, other genes have been found to be associated with PTS. Padiglia et al. (2010) found that PROP sensitivity is inversely related to salivary zinc ion concentrations and is associated with the SNP

rs2274333 (A/G) in Gustin. Gustin, also known as carbonic anhydrase VI (CA6), is a zinc metalloprotein (containing a zinc ion within the protein) secreted by glands (serous acinar cells of parotid, submandidular, and von Ebner) in the oral cavity (Fernley et al., 1995; Henkin et al., 1975; Leinonen et al., 2001; Parkkila et al., 1990; Thatcher et al., 1998). It was found that PSTs are associated with the A/A Gustin genotype; PNTs are associated with the G/G phenotype; and PMTs are associated with the presence of at least one A allele. It was suggested that this SNP, as the mechanism, affects function and taster status by influencing the binding ability of Gustin to zinc (Padiglia et al., 2010). Since Gustin requires zinc to be functional (Henkin et al., 1988; Law et al., 1987) and taste function is dependent on Gustin (Henkin et al., 1999a; Shatzman & Henkin, 1981; Stewart-Knox et al., 2008), if one of the genotypes results in the inability of zinc binding then taste function would be impaired, as was hypothesized by Padiglia et al. (2010).

In addition to its implications for taste function in the oral cavity, Gustin is also known to be a trophic factor for taste bud development (Henkin et al., 1999a; Henkin et al., 1999b). Since PROP taster status has also been associated with fungiform papillae density (Bartoshuk et al., 1994; Essick et al., 2003; Hayes et al., 2008; Tepper & Nurse, 1997), this SNP rs2274333 and other variations may be responsible for the reduced papillae counts found in PROP non-tasters and, consequently, their reduced oral chemosensory perceptions (Padiglia et al., 2010). Further research is needed to confirm this hypothesis.

Along with Gustin, Cabras et al. (2012) studied the composition and concentrations of salivary proteins for associations with PROP taster status. They demonstrated a strong association between PROP taster status and basal levels of specific

basic proline-rich salivary proteins. The study was performed by observing the compositions of PROP unstimulated and stimulated saliva. They found that both, the unstimulated and stimulated saliva from PROP super tasters, were associated with higher concentrations of the II-2 peptide and Ps-1 protein (Cabras et al., 2012). Cabras et al. (2012) proposed that PROP super tasters are capable of secreting these salivary proteins after stimulation, whereas PROP non-tasters cannot as a result of lacking a functional gene or genes. Interestingly, both of these salivary proteins are encoded by the PRB1 gene located on chromosome 12p13.2 (Azen et al., 1993). The PRB1 gene is subject to many variations that may be responsible for these associations with PROP taster status, although further genetic analysis is needed (Cabras et al., 2012).

As stated previously, PROP taster status has been found to be associated with fungiform papillae density. Taste buds, which contain taste receptors such as TAS2R38, are found mostly within fungiform papillae and circumvallate papillae (Arvidson, 1979). The number of taste buds within a single papilla varies widely from zero to twenty-seven with an average of 1.4 taste buds (Arvidson, 1979). Thus, the greater the number of papillae there are present on one's tongue, the greater the number of taste buds and receptors; therefore, a greater perception of a tastant would be expected. This means that the same TAS2R38 genotype carriers with different density of papillae can have different levels of sensitivity for a tastant. This was found by Miller and Reedy (1990) for sucrose, sodium chloride, and PROP solutions but not for quinine HCl and citric acid.

Much research has been performed to assess the impact of fungiform papillae counts on PROP taster status. Delwiche et al. (2001) found fungiform papillae counts influence only PROP perception for PROP tasters. They found that if more papillae were

stimulated, the subjects experienced a greater intensity whereas PROP non-tasters, regardless of the number of papillae stimulated, did not detect bitter taste, thereby indicating that a crucial component of the taste pathway for PROP detection is nonfunctional in PROP non-tasters (Delwiche et al., 2001). This nonfunctional component is likely the TAS2R38 genotype if it is the homozygous non-taster genotype (AVI) (Duffy et al., 2004; Kim et al., 2003). In addition to papillae counts influencing PROP taster status, Bartoshuk et al. (1994) found anatomical differences in the fungiform papillae by PROP taster status. PROP supertasters, on top of having more papillae, also had smaller papillae with more taste pores (small openings into the taste buds) (Bartoshuk et al., 1994). PROP intensity ratings were also found to be correlated with fungiform papillae density by other researchers (Bajec & Pickering, 2008). Therefore, it is evident that fungiform papillae density, as well as taste bud density is associated with PROP taster status, and should be included in future studies.

PROP taster status and its known associations with other attributes

PROP taster status has been found to be associated with a wide range of attributes/phenotypes including increased perceived orosensory sensations, motion sickness, food preference, fat perception, BMI, height of children, alcohol consumption, and alcoholism. These associations are outlined below.

Many cruciferous vegetables are known to contain compounds similar to PROP and PTC. These compounds are known as glucosinolates and are found in members of the Brassica, Raphanus, and Nasturtium geni, including radish, kale, and watercress (Fahey et al., 2001; Sandell & Breslin, 2006). Sandell and Breslin (2006) found that the

TAS2R38 genotypes associated with PTS are also associated with the bitterness perception of these glucosinolate-producing plants. Interestingly, glucosinolates are thyroid toxins; therefore, it is vital that they are detected prior to ingestion to prevent thyroid-related diseases.

Additionally, Benson et al. (2012) found a significant association between PROP taster status and vection-induced motion sickness. They found that PROP non-tasters experienced significantly greater degrees of motion sickness and nausea versus PROP medium and super tasters. The authors suggested the susceptibility to motion sickness and nausea has coevolved with non-tasters to maintain protection against potential toxin consumption while allowing for consumption of bitter compounds such as phytonutrients without the initial rejection in the oral cavity. This suggests that PROP non-taster status is a positive evolutionary selection mechanism.

PROP taster status has also been shown to be associated with fat perception, BMI, (Tepper & Nurse, 1997; Tepper & Ullrich, 2002) and food preference (Duffy, 2007; Tepper, 2008). Tepper and Nurse (1997) found PROP super tasters and medium tasters to be more discriminative towards fat content than PROP non-tasters, suggesting that PTS influences fat perception and could affect consumption. Additionally, Tepper and Ullrich (2002) demonstrated that PROP non-tasters and medium tasters have greater BMIs than PROP super tasters in subjects with low dietary constraints. On the other hand, other groups have shown a lack of association between PTS, BMI, fat perception (Duffy & Bartoshuk, 2000; Yackinous & Guinard, 2001), and food preference (Drewnowski et al., 2007). Yackinous and Guinard (2001) found no difference in the ability of subjects to discriminate fat content based on PROP taster status in a variety of

food types, contradicting findings by Tepper and Nurse (1997). Additionally, Duffy and Bartoshuk (2000) found that BMI was not associated with PROP taster status in both males and females. Lastly, Drewnowski et al. (2007) found that PTS was not associated with the dietary habits and food preferences of individuals for sweet and high fat foods. They also found no association between PTS and BMI (Drewnowski et al., 2007).

Furthermore, PROP taster status was found to be associated with height and choosy eating in children, with PSTs being shorter in stature and pickier eaters at age 10 than their PNT counterparts (Golding et al., 2009). In addition, Keller and Tepper (2004) found that PROP super tasting girls had significantly higher weight-to-height percentiles, whereas PROP super tasting boys had significantly lower weight-to-height percentiles compared to their PNT counterparts. The same associations were also found with body mass index (BMI). Therefore, there is significant evidence for both the association and lack of association between PROP taster status and dietary habits, food preference, and BMI, thus leading to the unanswered question of whether PTS is a factor in one's dietary habits and preferences.

In addition, PROP taster status has been linked to the consumption of alcohol (Duffy et al., 2004) and alcoholism (DiCarlo & Powers, 1998; Pelchat & Danowski, 1992) as a result of the bitter taste involved. Subjects with the TAS2R38 genotype typically associated with PROP non-tasters (AVI) were reported to have greater alcohol consumption than subjects with the other two common genotypes, PAV/AVI and PAV/PAV (Duffy et al., 2004). The same research also found PROP taster status to be strongly associated with perceived ethanol intensity, with PROP super tasters rating ethanol solutions with greater intensity than non-tasters (Duffy et al., 2004). This

relationship was also confirmed by Hayes et al. (2011), who found that the AVI homozygote carriers drink alcohol more frequently than the carriers of other genotypes. This indicates that the presence of one copy of the PAV haplotype is enough to depress alcohol consumption. These two studies suggest a potential genetic predisposition to greater alcohol consumption based on one's TAS2R38 genotype. Therefore, both PROP taster status and the genotypes associated with it are of great importance due to the impact on alcohol consumption, thereby on health.

As previously studied by Pickering et al. (2006), significant preference differences for the sweetness of wines were observed among PSTs, PMTs, and PNTs. This indicates PROP taster status is associated with the perception of other tastes as well. Additionally, PROP super tasters have been found to rate other oral stimuli in aqueous solutions as more intense than their PROP non-taster counterparts (Bajec & Pickering, 2008). However, due to the complexity of taste transduction and the vitality associated with it as outlined previously, it is unlikely that one classification (PTS) and the corresponding genes are solely responsible for these heightened intensity ratings. Hayes and Keast (2011) proposed that individuals who experience taste modalities at greater intensities be reclassified as hypergeusia instead of being referred to as super tasters, because the term super taster is typically associated with PROP perception.

Other TAS2R genes of interest

Since it is known that TAS2R38 cannot fully explain the PROP taste phenotypes, further research into other genes is necessary. Hayes et al. (2011) found SNPs within TAS2R3, TAS2R4, and TAS2R5 to be associated with the bitterness of espresso. They

also found a SNP within TAS2R19 to be associated with grapefruit-juice-liking and bitterness. Lastly, a SNP in TAS2R16 was found to be associated with alcohol intake, along with the TAS2R38 genotype (Hayes et al., 2011). Recently, another SNP, rs1015443, in TAS2R13 has been found to be associated with alcohol consumption (Dotson et al., 2012). With the increasing number of SNPs in bitter taste receptors found to be associated with alcohol intake, there is greater support for the association between bitter taste perception and alcohol consumption. A few of these SNPs were analyzed further in this study to investigate potential relationships with PROP taster status and thermal taster status.

Thermal taster status

Thermal taste is a phenomenon in which the stimulation of parts of the tongue with heating or cooling elicits a taste response, resulting in another taster classification, thermal taster status (TTS). Thermal taste was observed and defined by Cruz and Green (2000) using a thermode on isolated points of the tongue. However, the discovery of thermal taste was made as early as 1964 using different temperatures of water to evoke sweet and sour tastes on the whole tongue (von Bekesy, 1964).

In the current protocol, a thermode is heated and cooled by a water-circulated heat sink. The heating cycle consists of cooling to 15°C, followed by heating to 40°C, where it is held for 1 second. The cooling cycle consists of cooling to 5°C and holding that temperature for 10 seconds (Pickering, Moyes et al., 2010). Cruz and Green (2000) observed that upon heating, sweetness was sensed, and upon cooling, sourness was sensed. However, studies by Green and George (2004) identified that sweetness is the

most common taste resulting from thermal stimulation of the tongue, occurring in approximately 50% of thermal tasting individuals. Those individuals with a taste response evoked from thermal stimulation are classified as thermal tasters (TTs) and those who exhibit no taste sensations are classified as thermal non-tasters (TnTs) (Green & George, 2004).

Additionally, it has been observed that TTs tend to perceive other orosensory sensations as more intense (i.e. sweet, sour, bitter, salty, astringency, and overall flavor intensity) than TnTs in aqueous solutions (Bajec & Pickering, 2008; Green & George, 2004). This increased responsiveness was also found for wine and beer (Pickering, Bartolini et al., 2010; Pickering, Moyes et al., 2010). As a result of these discoveries, it is believed that thermal taster status may play a larger role in taste preference than previously thought (Pickering, Moyes et al., 2010). However, no difference has been observed between TTs and TnTs for oral chemesthetic perception (tested using capsaicin and menthol) on the lip and tip of the tongue (Green et al., 2005). This suggests that different factors and pathways may be involved in thermal taste stimulation from chemesthetic stimulation (Green et al., 2005). It is likely that thermal taste is the result of a temperature-sensitive pathway related to taste perception.

Interestingly, both PTS and TTS are associated with other oral sensations and preferences. This similarity between increased orosensory sensation sensitivities suggests a possible relationship between TTS and PTS, although previous phenotypic analysis suggests a lack of association (Bajec & Pickering, 2008; Bajec & Pickering, 2010; Drewnowski et al., 2007; Tepper, 2008). Therefore, genetic analysis of PTS and TTS is needed to confirm these previous findings.

The mechanism by which thermal taste occurs is not yet known, hence the interest in a potential gene or genes underlying thermal taster status in this study. It is proposed that thermal taste is signaled through a temperature-sensitive process and taste transduction (Green & George, 2004). Transient receptor potential melastatin 5 (TRPM5) is considered a gene of interest since it is a temperature-sensitive cation channel involved in taste transduction. Additionally, studies with knockout mice have suggested that TRPM5 is partly responsible for thermal taste (Talavera et al., 2005).

TRPM5 and thermal taste

TRPM5 is a monovalent-specific nonselective cationic channel that is voltage gated (Liu & Liman, 2003; Prawitt et al., 2003) and is activated by Ca^{2+} (Damak et al., 2006). TRPM5 is involved in the propagation of sweet, bitter, and umami taste (Zhang et al., 2003). It consists of six transmembrane domains with a short N-terminus and a long C-terminus (Palmer, 2007) and has been identified to function as a tetramer (Owsianik et al., 2006). It is proposed, based on studies of TRPM4, that the cation channel is located between the fifth and sixth transmembrane domains because TRPM5 and TRPM4 exhibit homology (Owsianik et al., 2006). Homology to TRPM8 is also evident (McKemy et al., 2002). Additionally, the voltage-sensitive domain of the TRPM5 receptor is proposed to be the fourth transmembrane domain based on studies of potassium ion channels (Liman, 2007).

TRPM5 transports sodium, potassium, and caesium but not calcium (Prawitt et al., 2003), and it is regulated by voltage, phosphoinositide concentrations, and temperature. It is also inhibited by low pH (Liman, 2007). TRPM5 is stimulated by signals from second messengers via other taste receptor pathways (TAS1R or TAS2R) or

changes in the voltage of the cell as a result of signaling from other taste receptors. It is also suggested that protein lipase C (PLC) mediates the pathway and signals TRPM5 (Liman, 2007). Stimulus from a PLC-mediated pathway could come in many forms, such as IP_3, diacyl glycerol (DAG), or the release of Ca^{2+} into the cell (Liman, 2007). This stimulus results in the activation of the TRPM5 channel and is believed to be involved in biochemical changes in the cell, such as the release of calcium into the cytosol. This results in electrical signal production and nerve signaling (Liman, 2007). The TRPM5 activation and transduction pathway is outlined in Figure 3.

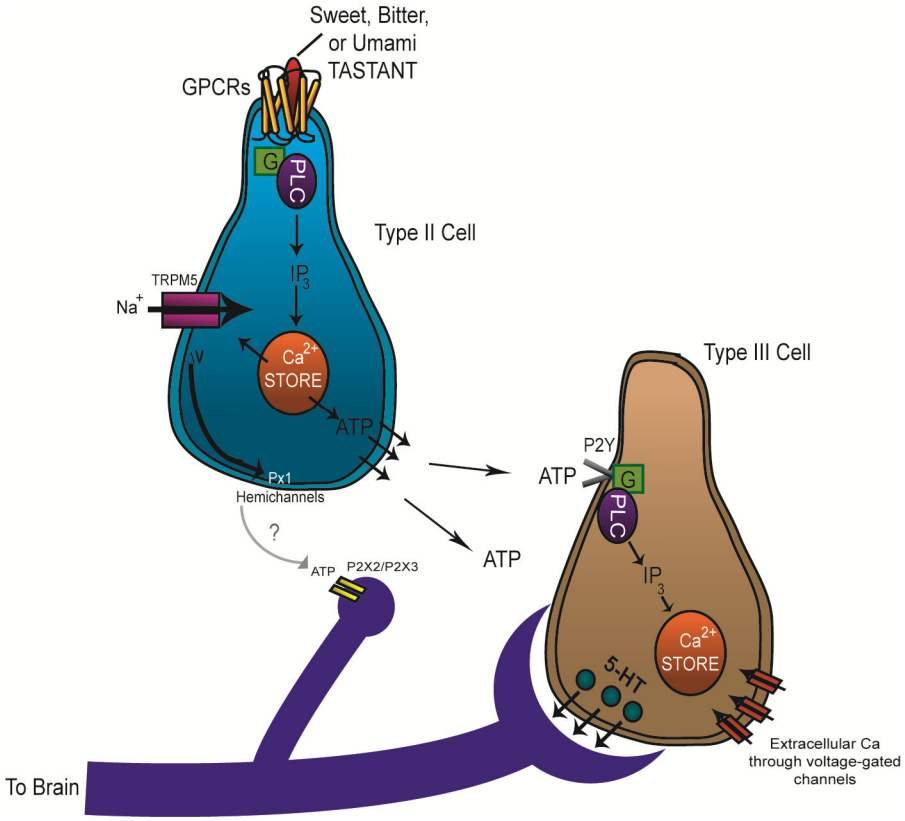

Figure 3: Bitter taste transduction pathway. Stimulus of a bitter, sweet or umami compound on the TAS1R or TAS2R receptor in the Type II taste cell, resulting in increased intracellular calcium ions signaling TRPM5, leading to greater calcium ion release and change in voltage, resulting in stimulation of the Type III taste cell and neuronal signaling (adapted from Palmer, 2007).

TRPM5 is believed to be involved in sweet, umami, and bitter taste based on mice knockout (KO) studies in which TRPM5 KO mice showed significantly decreased responses to sweet, bitter, and umami compounds (Damak et al., 2006) studied through the use of gustatory nerve recordings, initial lick responses adn 24 hour preference tests. A higher threshold for bitter compounds was observed in the KO mice in comparison to the wild-type mice, 3mM versus 0.1mM quinine respectively (Damak et al., 2006). Additionally, decreased responses in the glossopharyngeal nerve were observed following consumption of bitter compounds in the KO mice (Damak et al., 2006). This same diminished response to sweet compounds was observed in the KO mice with the wild-type mice preferring sucrose concentrations of 20mM versus 150mM for the KO mice (Damak et al., 2006). A significant decrease and complete absence (for synthetic sweet compounds sucralose and D-tryptophan, respectively) of stimuli in the chorda tympani nerve in response to sweet compounds was observed in the KO mice (Damak et al., 2006). It is evident based on this KO study that TRPM5 is involved in sweet, bitter, and umami taste because decreased responses were observed in the TRPM5 KO mice.

TRPM5 is expressed in approximately half of the taste receptor cells in the anterior and posterior of the tongue (Damak et al., 2006), further supporting TRPM5's involvement in sweet, bitter, and umami taste transduction. TRPM5 is also very sensitive to temperature; therefore, it is believed to be linked to thermal taste (Kaske et al., 2007). However, it has been indicated that heat alone is not enough to stimulate a response from the TRPM5 receptor (Liman, 2007) because an increase in calcium ions in the cells has been identified as a requirement for TRPM5 stimulation (Talavera et al., 2005). TRPM5 has a known functional range of 15°C to 35°C. An increase in sweet taste perception in

mice has been found within this functional range (Talavera et al., 2005). This is also the temperature range used for the heating cycle of thermal taste evaluation (Pickering, Moyes et al., 2010), hence the inclusion of TRPM5 in this study.

Additionally, TRPM5 has been found in olfactory cells through green fluorescence tagging (Kaske et al., 2007). This also supports the link between the TRPM5 gene and thermal taste because thermal tasters have been observed to have stronger senses of smell with increased responses to retronasal stimulation of vanillin in thermal tasters versus non-thermal tasters (Green et al., 2005) as well as ortho-nasally (Bajec & Pickering, 2008).

The TRPM5 gene is located on chromosome 11 and is highly conserved across many species. In humans, the TRPM5 gene is composed of 24 exons, with an open reading frame of 3,495 base pairs, resulting in a protein composed of 1,165 amino acids (Liman, 2007). Orthologs for TRPM5 have been identified in many species, including *Mus musculus* (mouse), *Gallus gallus* (chicken), *Danio rerio* (zebra fish), and *Tetraodon nigroviridis* (spotted green puffer fish) (Dünzinger et al., 2007). Additionally, through the use of HomoloGene on NCBI, orthologs for TRPM5 were also found in *Pan troglodytes* (common chimpanzee), *Canis lupus familiaris* (dog), and *Rattus norvegicus* (rat). Conservation of TRPM5 can be seen as far as primitive metozoa animals such as *Trichoplax adhaerens* (Zhu et al., 2010), indicating its importance to survival.

In addition to single nucleotide polymorphisms, the TRPM5 gene contains multiple repeat domains (UCSC Genome Browser, 2012), which may be potentially associated with the functionality of the TRPM5 protein. These repeat domains could affect the signal transduction pathway and possibly be associated with thermal taste. In

addition to the presence of multiple repeat domains, TRPM5 is subjected to copy number variation susceptible regions. Among five reported CNV cases involving TRPM5, two result in loss of the region spanning the entire gene and three result in gains in regions of the gene (Conrad et al., 2009; Jakobsson et al., 2008; Park et al., 2010). Copy number variations are defined as segments of DNA one kilobase (kb) or greater that have been duplicated or deleted compared to the reference genome (Redon et al., 2006). These copy number variations, if present, could result in the loss or decrease of function of the gene due to loss of exons or reading frame shifts. They could also result in higher expression of TRPM5. It is hypothesized that loss of function or decrease of function of the TRPM5 protein may be responsible for thermal non-tasters, whereas thermal tasters would exhibit a functional TRPM5 protein. Therefore, examination of copy number variation for TRPM5 was included in this study to examine potential associations with thermal taster status.

Hypotheses and Objectives

This study focused on the genetic contribution of TAS2R38, Gustin, other TAS2R genes, and TRPM5 to the taste phenotypes, which include PROP taster status, thermal taster status, and orosensory sensation intensity ratings. Based on previous research (Cabras et al., 2012; Calņ et al., 2011; Duffy et al., 2004; Padiglia et al., 2010; Talavera et al., 2005), we hypothesized that TAS2R38 and Gustin contribute to PROP taster status, while TRPM5 may contribute to thermal taster status. The objectives of this study were to test the contribution of these genes to PROP and thermal taste status by performing genotyping of variations in these genes for samples from individuals whom collected

phenotype data on taste was readily available, followed by association statistical analysis between the genotype and phenotype data.

SECTION II: METHODS

PART A: Sensory analysis

Subjects

The study consisted of 60 participants, 20 of whom were recruited using the SONA website (http://brocku.sona-systems.com/Default.aspx?ReturnUrl=%2f). SONA is a Psychology Research Pool website with the Department of Psychology at Brock University that allows participants to register for studies and receive research participation hours towards class requirements. Each participant was given a one-hour participation credit towards their first-year psychology course, following completion of the study. The remaining 40 participants were recruited and tested by researchers in the Pickering lab. In both cases, participants were 18 to 61 years old, with 41 females and 19 males and a mean age of 25.52 years ± 9.33 SD, all of whom were non-smokers. The Brock University Research Ethics Board approved all procedures (Brock REB file 09-258), and written consent was obtained from all subjects (Appendix VII). To establish the ethnic origin of the subjects the Census Canada "Ethnic Origin User Guide" (Statistics Canada Census Ethnic Origin User Guide, Statistics Canada Catalogue no. 92-403-GIE) was used. Of the 60 subjects, 53 subjects were Caucasian (reporting "White" as their ethnicity) and the remaining 7 were non-Caucasian.

Scale usage and acclimation

Paper versions of the quasi-logarithmic general Labeled Magnitude Scale (gLMS) were used to collect thermal taste and PROP intensity ratings (Bajec & Pickering, 2010; Bartoshuk et al., 2003; Bartoshuk et al., 2004) (Appendix XI and XII), and a generalized

Visual Analog Scale (gVAS) was used for training with the orosensory sensations (Pickering, Moyes et al., 2010) (Appendix IX). The quasi-logarithmic gLMS is a scale with a bottom anchor of "no sensation" (0mm) and "strongest sensation imaginable" as the top anchor (100mm), and with labels in between of "barely detectable" (1.4 mm), "weak" (6 mm), "moderate" (17 mm), "strong" (35 mm), and "very strong" (53 mm) (Bajec & Pickering, 2008; Bartoshuk et al., 2004; Green et al., 1993; Pickering, Moyes et al., 2010). The quasi-logarithmic gLMS scale has been found to provide clear separation between the PROP taster status classifications (Bartoshuk et al., 2003). The gVAS scale has a bottom anchor of "no sensation" (0mm) and a top anchor of "strongest sensation ever experienced" (100mm), with three unlabeled equidistant line anchors at 25, 50, and 75mm. Subjects received verbal and written instructions that the top of the scale represented the most intense sensation in any modality that they could ever imagine experiencing and were told to think of experiences from a variety of different modalities to assist in understanding the general nature of the scale (Bartoshuk et al., 2003).

Each individual was asked to rate the intensity of 15 remembered sensations on the gVAS scale to familiarize themselves with the scale (Green & Hayes, 2004; Porubcan & Vickers, 2005) and again with the gLMS scale (Appendix VIII). The 15 remembered sensations were bitterness of black coffee, brightness of the sun when looking directly at it, burn of cinnamon gum, burning sensation from eating a whole hot pepper, coolness of a peppermint candy, coolness of an ice-cold beverage, heat of drinking a hot tea, pain from biting your tongue, saltiness of ocean water, sourness of a lemon, sweetness of cotton candy, tingling sensation of a pill on the tongue, and the warmth of sipping lukewarm water.

Overall procedure

Each subject attended a one-hour session. All testing was performed in the order listed below. Each test was separated by approximately 5-minute intervals in which subjects completed questionnaires (Appendix XIII-XVI) . All oral solutions were made with pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA), stored in the dark at 3–4°C and were kept for no more than 5 days. Solutions were brought to room temperature (22°C±2) prior to testing.

Prototypical tastants and astringent

Each participant was trained with stimuli eliciting the basic tastes: sweet, sour, bitter, and umami, and astringency for mouth-feel. The compounds used to elicit the tastes and mouth-feel are listed in Table 1. Training was performed to ensure all subjects were capable of detecting and differentiating between each taste/mouth-feel and to ensure more accurate ratings for thermal taste testing.

Table 1: Compounds and concentrations used for each taste/mouth-feel solution in taste training.

Compound	Concentration (mM)	Source	Sensation elicited
Sucrose	250	Sigma-Aldrich #028K0004	Sweet
Citric acid anhydrous	3.25	Fisher Scientific #070975	Sour
Quinine monohydrochloride	2.75e−8	SAFC #20296CJ	Bitter
Aluminum sulfate	8.77e−7	Sigma-Aldrich #046K0058	Astringent
L-glutamic acid monosodium salt hydrate	125	Sigma-Aldrich #076K12671	Umami

Each participant was given 20ml of each orosensory sensation eliciting solution in random order in ISO (International Organization for Standardization) tasting glasses

labeled with their contents. Participants were instructed to take the full volume and rinse well for 10 seconds, expectorate, and wait approximately 10 seconds before recording the peak intensity of the taste/mouth-feel for each solution. All ratings were performed using a gVAS scale. A minimum 1-minute rest was taken between each sample, and participants were encouraged to take longer if needed. During this rest, subjects were required to take a pectin rinse (5g/L; Pomona's Universal Pectin, Massachusetts, USA), followed by filtered water rinses (Brita, Ontario, Canada) to reduce carryover effects. Filtered water and pectin were at room temperature (22°C ± 2). Additionally, filtered water was available to participants *ad libitum*. All solutions were tested in duplicate. The second round of solutions were triplicate-coded, and participants were asked to identify the taste/mouth-feel (Appendix X). If identifications were incorrect, participants were informed of their errors, and they repeated tasting of the solutions for a maximum of two times. Participants were given verbal instructions for astringency, that it is a drying and constricting sensation typically associated with black tea and red wines (Bajec & Pickering, 2008) .

6-n-propylthiouracil (PROP)

6-n-propylthiouracil (PROP) was used as the bitterant since it is a proxy for taste responsiveness and known to associate with the TAS2R38 gene. A 3.2mM solution of PROP was made by dissolving PROP (MP Biomedicals, Ohio, USA) in pure water on low heat using a magnetic stirring plate. PROP testing was performed using 10ml of the 3.2mM PROP solution. Each participant was instructed to take the full volume and swish

for 10 seconds, expectorate, and then wait 10 seconds prior to rating the peak intensity of bitterness experienced using the gLMS scale.

Thermal taste testing

Thermal taste testing was performed as described in Bajec and Pickering (2008). Briefly, a 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode) was applied to the subject's extended tongue by the researcher. For hygienic purposes, the thermode was wrapped in a clean piece of plastic wrap (SC Johnson, Wisconsin, USA) for each participant. Five locations were tested: the palm of the hand, the edge of the middle of the bottom lip, and three locations of the tongue: tip and 1cm left and right of the tip. Two cycles (heating and cooling) were performed in duplicate with a break between reps. Heating cycles consisted of cooling the tongue from 35°C to 15°C, warming to 40°C, and holding for 1 second at 40°C. Cooling cycles consisted of warming to 35°C, followed by cooling the probe to 5°C, and holding for 10 seconds at 5°C. Participants were instructed prior to testing that some people do taste something and others do not and that they should indicate a taste experienced only if they were confident they tasted something. They were not instructed as to which tastes are typically experienced. Each participant was asked to rate temperature, sweet, bitter, sour, salty, and other taste sensation for each location of the tongue and they were asked to identify the taste. For the palm and lip, participants were asked to rate temperature only. All warming trials were performed prior to cooling trials to prevent temperature adaptation due to cold stimulation (Bajec & Pickering, 2008; Bajec & Pickering, 2010; Green & George, 2004) .

Classification of taster status

Ratings from the basic taste and astringency trials were assessed for potential associations with the genotype data. Since each subject rated the attributes twice, these values were averaged and normalized using their brightness of the sun ratings from the scale acclimation procedure. Individual sun brightness ratings were divided by the average sun brightness rating for all subjects, resulting in a normalization factor, which the individual taste/astringency ratings were divided by (Bajec & Pickering, 2010). This normalization allowed for comparison of perceived intensities across subjects by rescaling the oral stimuli ratings by a non-taste sensation. Direct comparison of perceived intensities was considered inappropriate since all of the subjects had different experiences affecting their use of the scales. These normalized ratings for sweet, bitter, umami, and sour taste along with astringency were statistically analyzed for potential associations with the genotypes studied.

Next, PROP taster status categories were defined, based on their ratings on the gLMS scale, as PROP non-tasters (PNT, <10.9 mm), PROP medium tasters (PMT, 10.9–61.5 mm), and PROP super tasters (PST, >61.5 mm) (Bajec & Pickering, 2010; Porubcan & Vickers, 2005). In order to effectively compare the perceived intensity of PROP bitterness across all subjects, PROP ratings were normalized using the subject's brightness of the sun rating as described above. Normalized taster statuses were determined using the same cut-off values as non-normalized PTS. Additionally, the strength of association between non-normalized phenotypes (PROP taster status and raw ratings) and genotypes was also studied to determine the strongest statistical association.

Thermal tasters were defined as rating the same taste (sweet, sour, bitter, or salty) at the same location above "weak" on the gLMS scale for both reps. Thermal non-tasters were defined as rating all tastes at "no sensation" for both reps. All other participants who could not be defined as thermal tasters or thermal non-tasters were considered "non-classifiable" and were not used for the analysis of thermal taste (Bajec & Pickering, 2010; Green & George, 2004). Ratings for thermal taster classification were not normalized in order to maintain the "weak" position on the gLMS (Bajec & Pickering, 2010).

There were two cohorts of subjects from the studies. The Cohort 1 samples were selected for identifying the differences between the four phenotypic groups (TT and PST, TT and PNT, TnT and PST, and TnT and PNT); therefore, PMTs were excluded from this portion of the study. The second cohort (Cohort 2) was aimed at studying all PROP taster statuses along with TTs and TnTs, and they were chosen based on TTS alone without bias for any type of PTS. Statistical analysis was performed for each cohort individually for all statistical tests performed in this thesis, and since no significant differences were evident between the cohorts for the associations examined, they were combined as one set to increase the sample size.

PART B: Genetic analysis

Sample collection and DNA extraction

DNA samples were collected using Scope ™ Original Mint Mouthwash (Procter & Gamble Inc; Toronto, Ontario). At the end of the study session, each participant was given 10ml of mouthwash in a 50ml plastic conical tube. Participants were instructed to take the full volume into their mouth and swish vigorously for 1 minute, which was timed by the researcher, then expectorate the mouthwash back into the conical tube. Samples were placed in a –80°C freezer within 30 minutes of sample collection for Cohort 2. Cohort 1 samples were placed in a frost-free freezer (approximately –20°C) within 30 minutes of sample collection.

To extract the DNA, samples were centrifuged at 4000 rpm for 30 minutes at 4ºC in an IEC Centra-8R Centrifuge (International Equipment Company; USA), and the pellet was resuspended in 1.0ml of wash buffer (10mM EDTA, 10mM Tris-HCl [Mediatech Inc], 1% NaCl, pH 8.0). Samples were then centrifuged at 4000 rpm for 5 minutes in a Microfuge®18 Centrifuge (Beckman Coulter; Germany) and the final pellet was resuspended in 250µl TE buffer (10mM EDTA, 10mM Tris-HCl, pH 8.0). The DNA extraction was performed using a Saliva DNA Isolation Kit (Norgen Biotek Corp; St. Catharines, Ontario) according to the manufacturers' directions. The obtained DNA samples were stored at –20ºC before use.

SNP selection and primer design

Single nucleotide polymorphisms (SNPs) were chosen based on the 1000 genome project data (Altshuler et al., 2010) and the functional type of SNP obtained from the

Single Nucleotide Polymorphism database (dbSNP) (Sherry et al., 2001). Non-synonymous coding SNPs with minor allele frequencies (MAF) in a relevant population (Caucasian/European) greater than 10% were chosen. The MAF requirement was set to 10% to correspond to the phenotypic frequencies of PTS and TTS. To further narrow down the SNPs, multiple protein sequence alignments with other primate species and other species (*Homo sapiens*, *Pan troglodytes*, *Macaca mulatta*, *Gorilla gorilla*, *Canis lupus familiaris*, *Bos taurus,* and *Mus musculus*) were performed using ClustalW (Larkin et al, 2007) (Appendix VI). SNPs located in regions corresponding to the protein-coding regions that were conserved across other species with MAF of 10% were chosen and primers were designed for each SNP for PCR amplification. In some cases, multiple SNPs were amplified in one reaction depending on the location within the gene. The SNPs selected to be studied can be found in Table 2.

Table 2: SNPs chosen for analysis, their genomic and protein locations, minor allele frequencies (MAF), nucleotide changes and amino acid (AA) substitutions for non-synonymous SNPs.

SNP	Gene	Genomic Location	Allele Change	MAF*	AA Location and Change
rs2274333	Gustin	chr1:9017204	A/g	0.32	Ser90Gly
rs1204064	TAS2R16	chr7:104971734	C/t	0.06	Non-coding
rs860170	TAS2R16	chr7:122635024	A/g	0.29	Arg222His
rs846664	TAS2R16	chr7:122635173	G/T	0.08	Asn172Lys
rs2233989	TAS2R16	chr7:122635229	c/T	0.03	Leu154Leu
rs978739	TAS2R16	chr7:122635900	A/g	0.37	non-coding
rs2233998	TAS2R4	chr7:141478308	c/T	0.47	Phe7Ser
rs2234001	TAS2R4	chr7:141478574	C/g	0.47	Val96Leu
rs10246939	TAS2R38	chr7:141672604	C/T	0.45	Ile296Val
rs1726866	TAS2R38	chr7:141672705	C/T	0.41	Val262Ala
rs713598	TAS2R38	chr7:141673345	C/G	0.47	Ala49Pro
rs79526258	TRPM5	chr11:2426259	C/t	0.01	Gly1136Ser
rs34364959	TRPM5	chr11:2432666	C/t	0.07	Gly900ser
rs34350821	TRPM5	chr11:2438963	a/C	0.07	Val335Leu
rs3986599	TRPM5	chr11:2439542	C/t	0.19	Val254Ala
rs886277	TRPM5	chr11:2439767	a/C/g/t	0.47	Asn235Ile,Ser,Thr
rs61741881	TRPM5	chr11:2441589	C/t	0.03	Gly171Asp
rs111504104	TRPM5	chr11:2442282	c/G	N/A	Arg149Gly
rs1015443	TAS2R13	chr12:11061122	c/T	0.46	Asn259Ser
rs10772420	TAS2R19	chr12:11174276	A/g	0.40	Arg299Cys
rs1868769	TAS2R19	chr12:11174753	A/g	0.25	Leu140Leu
rs12578654	TAS2R19	chr12:11174854	c/T	N/A	Cys106Tyr
rs4763235	TAS2R19	chr12:11175414	C/g	0.40	non-coding
rs10845293	TAS2R44	chr12:11183255	A/g	0.44	Ala227Val

* MAF data is from the 1000 genome project; all data was collected from dbSNP.

Primers were designed based on genomic sequences retrieved from the UCSC Genome Browser (Kent et al., 2002) for the region containing the chosen SNPs using the Primer3 online tool (Rozen & Skaletsky, 2000). The following requirements were used for primer design: GC content of 40–70%, melting temperature of 57–63°C, and product size (dependent on the number of SNPs within the desired amplicon). The PCR products ranged from 200 to 1000 base pairs (bp). The primers used for each SNP are listed in

Table 3 with the corresponding product size, melting temperature (Tm), and the experimentally determined annealing temperature.

Table 3: Primers used for each SNP and the melting temperature (Tm), product size in base pairs (bp) and the annealing temperature.

Primer Name	Gene	Primer Sequence	Size (bp)	Tm °C	Annealing Temp °C
rs111504104_F	TRPM5	ACGAGGCCCTCTTCCATC	262	60	63
rs111504104_R	TRPM5	CTGCCCAAACCCTTCTGAG		58	
rs79526258_F	TRPM5	CTGGTTCCCACCGCTCAG	300	63	63
rs79526258_R	TRPM5	GTGAGGGTCTGTGGTGAGG		63	
rs61741881_F	TRPM5	AGGGCTCAGCCTCGTCTC	260	61	63
rs61741881_R	TRPM5	ACTGGGTCCCAAGGCTTC		61	
rs34364959_F	TRPM5	CGCTGACGTCCCTCTCTCT	264	61	63
rs34364959_R	TRPM5	CACACAGGGCTCCAATTTCT		61	
rs886277_F	TRPM5	GCCTGAGAGCCTGAGATCC	999	60	63
rs886277_R	TRPM5	AGCGGCTCTGAGCATGAAG		58	
TAS2R13_F	TAS2R13	CCATGGACACTCAAGGATG	1032	57	62
TAS2R13_R	TAS2R13	TGTGGTCTGAATGGCTTATGA		59	
TAS2R16_F	TAS2R16	GCTTCCAGAGAGAGGGGTTT	1045	60	62
TAS2R16_R	TAS2R16	TCAATTGCTCGGGAGTCTTT		60	
TAS2R19_F	TAS2R19	GATGCTCCCCTTGTGAATCT	987	59	62
TAS2R19_R	TAS2R19	CAAGTGTTACTAAGCCTGCATTTT		59	
TAS2R31_F	TAS2R31	GAAGTCAGGGAGACCACGAA	1047	60	62
TAS2R31_R	TAS2R31	AGAAGACACACAATGCCCCT		60	
TAS2R4_F	TAS2R4	TCTTCTGCCTCCACTATCAGC	1028	60	55
TAS2R4_R	TAS2R4	CACTGGGAAAACTGCAAAC		57	
TAS2R38_F*	TAS2R38	GCTTTGTGAGGAATCAGAGTTGT	1196	60	62
TAS2R38_R*	TAS2R38	GAACGTACATTTACCTTTCTGCACT		60	
Gustin_F	Gustin	TGACCCCTCTGTGTTCACCT	251	61	61
Gustin_R	Gustin	GTGACTATGGGGTTCAAAGG		57	

*Primers were taken from (Kim et al., 2003).

Following primer design, primers were tested in the UCSC In-Silico PCR program (Kent et al., 2002) to ensure no repeat regions, SNPs, or other genomic variations were present within the primer binding regions. If a variation was present, different primer pairs were chosen. Primers were ordered from either Integrated DNA Technologies (IDT) (California, USA) or Alpha DNA (Quebec, Canada).

PCR amplification and gel electrophoresis

All PCR amplifications for the SNP analysis were performed using Invitrogen AccuPrime reagents (Invitrogen Corp; Carlsbad, California) and performed on either an Eppendorf Mastercycler or Eppendorf Mastercycler gradient. The PCR conditions were optimized for each primer individually through the use of gradient PCR. Gradient PCR allows for testing of a range of annealing temperatures to determine the optimal conditions with minimal nonspecific product formation.

PCR products were visualized using agarose (Invitrogen Corp; Carlsbad, California) gel electrophoresis with either RedSafe™ (FroggaBio Inc; Toronto, Ontario) or Ethidium bromide (Invitrogen Corp; Carlsbad, California) staining on 1% gels. Visualization of the products was performed using ultraviolet (UV) light exposure and Gel Doc™ software (Bio-Rad Laboratories Ltd; Mississauga, Ontario).

PCR purification and sequencing

For DNA sequencing, PCR products were purified using the Norgen Biotek Inc PCR purification kit (Norgen Biotek Corp; St. Catharines, Ontario) according to the manufacturers' directions, with the eluted sample run through the column twice to increase yield. DNA concentrations were determined using the GE NanoVue (GE Healthcare Life Sciences; Pittsburgh, USA) in ng/ul. Sequencing preparation was performed according to the Center for Applied Genomics (TCAG, Toronto, ON) requirements: 5pmol of primer in 0.7ul and the required DNA concentration depending on product size in 7ul, which can be found in Table 4.

Table 4: DNA concentrations for sequencing at TCAG for PCR product ranges in kilobase (kb).

PCR product size	Required DNA (ng)
1-2kb	50 to 100
<1kb	50
<500bp	20

For many of the SNPs studied, some samples were not sequenced for a few reasons. First and foremost were the costs associated with sequencing. Initially, a few subjects with the taste phenotypes at the two extremes (for example PSTs and PNTs) were chosen for sequencing to determine if a variation between the subjects was evident. If both taster groups had relatively the same genotype, these SNPs were not tested further due to the assumed lack of an association; therefore, there was no justification for the cost of sequencing. Additionally, many of the DNA samples collected had very low yields, possibly due to the method used for collecting the DNA and/or long-term storage in a frost-free freezer resulting in degraded DNA. The low yields of these samples prepared in the beginning of the project resulted in larger volumes of DNA being used in PCR reactions compared to those of higher yields. This ultimately led to the complete consumption of these samples before the end of the project. Consequently, further sequencing of these individuals was not possible without additional sample collections, which was not possible. These errors could be corrected easily in future studies through the use of proper storage techniques upon sample collection along with a more efficient DNA extraction method, resulting in higher yields.

Sequence analysis

Sequencing was performed on an ABI 3730XL (Applied Biosystems, Carlsbad, California) at The Centre for Applied Genomics (TCAG, Toronto, Canada). Sequences were accessed via the TCAG website and downloaded as .fsa files and viewed in FinchTV (Geospiza Inc, Seattle, Washington). All SNP data was stored in Microsoft Excel (Microsoft, California, USA) along with phenotype data. Submission of the sequences to GenBank (Benson et al., 1997) is currently in progress.

Statistical analysis

All statistical analyses were performed in SAS 9.2 (SAS Institute Inc, North Carolina, USA). Regression analysis (proc reg) and Pearson's correlation analysis (proc corr) were used to study the relationship between genotype and phenotype. The Bonferroni correction was used when multiple SNPs were assessed in one analysis. Dummy codes were used to create categorical data for PROP taster status, thermal taster status and genotype, as shown in Table 5.

Table 5: Dummy codes for the statistical analysis of PROP taster status (PTS), thermal taster status (TTS) and the allele of each SNP.

	Code		
Attribute	0	1	2
PTS	PNT	PMT	PST
TTS	TnT	TT	
Allele	Homozygous Ancestral Allele	Heterozygous	Homozygous Minor Allele

Multiplex PCR primer design and optimization

Multiplex PCR, upQMPSF (quantitative multiplex PCR of short fluorescent fragments), was used to detect copy number variations in TRPM5 and the surrounding regions. Multiplex PCR primers were designed using FastPCR (PrimerDigital Ltd, Finland) to ensure compatibility among all primers, with a 20bp universal sequence to which a universal primer could bind. The universal forward primer had a fluorescent probe (Fluorescein 6-FAM) attached to the 5' end to allow for easy detection via capillary electrophoresis (CE). A diagram explaining the procedure can be found in Figure 4.

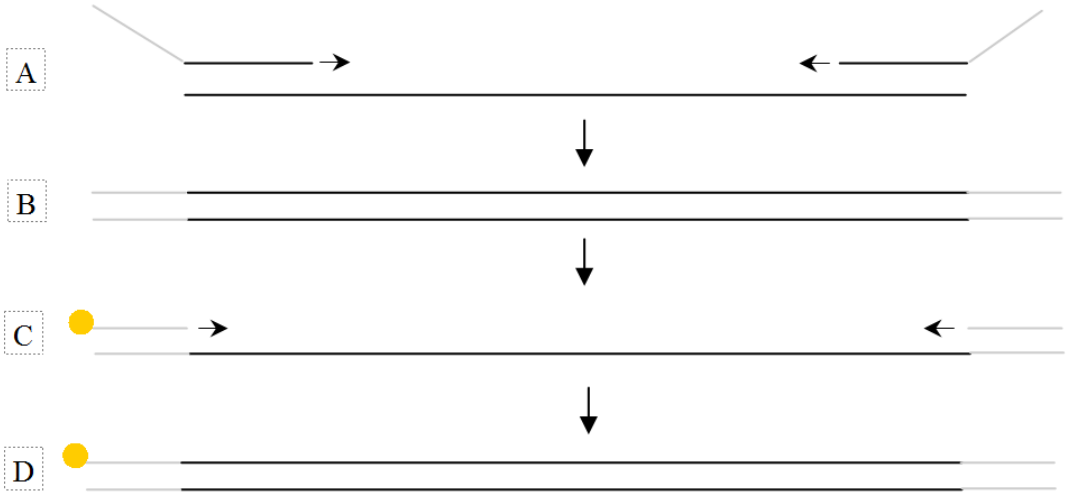

Figure 4: Schematic diagram of multiplex Polymerase Chain Reaction for TRPM5 copy number variation analysis.
(A) Reaction 1 (R1) annealing and extension of site-specific primers with unique sites. There were eight of these pairs. The grey line represents the universal primer sequence (identical on all eight pairs) attached to the site-specific primer. (B) R1 PCR products of eight different sequences. (C) Reaction 2 (R2) annealing and extension of universal primers with eight different amplicons. The forward universal primer to the left of the diagram has a fluorescent probe (Fluorescein 6-FAM) attached to the 5' of the primer indicated by the yellow circle. (D) R2 PCR product with attached Fluorescein probe, which can be detected using capillary electrophoresis.

The multiplex PCR was optimized by adjusting primer concentrations in the reaction to result in the production of approximately equal proportions of each fragment. The multiplex PCR set was designed to have three control regions outside of copy number subjected regions and five primers pairs within the copy number regions, two of which were within exons of TRPM5 (exons 3 and 22). The primers and their locations within the genome can be found in Table 6, and an image of the locations of each of the primers can be found in Figure 5.

Table 6: Multiplex PCR primers for TRPM5 and their corresponding Tm and product size.

Primer Name	Primer Sequence	Size (bp)	Tm °C
3q_uR2_138F	tgatgtgcaactatgtctcgTcgtgtatgcccagcattag	138	79
3q_uR2_138R	agtcctggtactgaatgatcTgcacactcgagggcaagtg	138	81
TRPM5_3_2222920-3150F	tgatgtgcaactatgtctcGctggggcttagccatggtagg	228	69
TRPM5_3_2222920-3150R	agtcctggtactgaatgatCgactggacagcacacagctcc	228	68
11q_uR1_240F	tgatgtgcaactatgtctcgCctccatgacaggacacagc	280	68
11q_uR1_240R	agtcctggtactgaatgatcGtgagctcccaggcatgagg	280	69
TRPM5_4_2453225-600F	tgatgtgcaactatgtctcGcgtgtctctgtgtgagcactg	351	68
TRPM5_4_2453225-600R	agtcctggtactgaatgatCcggggccaactgtagcaaag	351	68
TSPAN32_2334258-700F	tgatgtgcaactatgtctcGcaggcagcacatccagccag	444	70
TSPAN32_2334258-700R	agtcctggtactgaatgatCcacacaatggccgtcagagtg	444	68
TRPM5_E3F	tgatgtgcaactatgtctcGttccctatgcggtactgccac	468	68
TRPM5_E3R	agtcctggtactgaatgatCtggctgctgattgggcctag	468	68
KCNQ1_2469371-853F	tgatgtgcaactatgtctcGtgctgtgagccttagccagg	480	68
KCNQ1_2469371-853R	agtcctggtactgaatgatCgctgcagaacaactgcccag	480	68
TRPM5_E22F	tgatgtgcaactatgtctcGaaggcgaggggtcttcaatg	504	67
TRPM5_E22R	agtcctggtactgaatgatCagatctgggcatgagggagg	504	67
Universal_F	tgatgtgcaactatgtctcg	N/A	56
Universal_R	agtcctggtactgaatgatc	N/A	51

*The capital base in the primer sequence indicates the beginning of the site-specific sequence; the sequence before the capital base is the universal sequence to which the universal primers will anneal in R2.

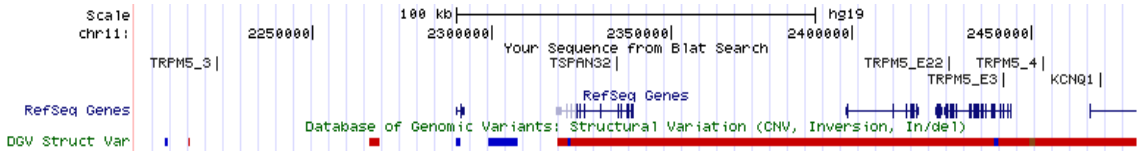

Figure 5: UCSC Genome Browser with blat sequence searches for each primer location for the TRPM5 multiplex reaction and reference sequence (RefSeq) genes. The following primers are located within this image TRPM5_3, TSPAN32, TRPM5_E22, TRPM5_E3, TRPM5_4, KCNQ1. Along with the primer locations, the copy number variation regions are indicated from the Database of Genomic Variants, with loss CNVs indicated in red and gain CNVs indicated in blue.

For ease of reading, primers TRPM5_3 and TRPM5_4 were renamed as Control1 and Marker1 respectively, because they are not located within the TRPM5 gene. Each multiplex reaction consisted of two steps. The first step was amplification of the target sequences using a mixture of the eight primer pairs. The conditions for this reaction (R1) were as follows:

95°C 5minutes
94°C 1minute
59°C 30 seconds **4 cycles**
68°C 45 seconds
94°C 1 minute *8 cycles*
68°C 1 minute
68°C 10 minutes

Following R1, 1µl of R1 was placed in a second PCR reaction (R2) with the universal primer and run at the following conditions:

94°C 37 seconds
50°C 30 seconds **20 cycles**
68°C 45 seconds
68°C 50 minutes

Capillary electrophoresis and analysis of copy number variation

Of each sample, 10µl was sent in eight-strip PCR tubes for capillary electrophoresis fragment analysis to TCAG on an ABI3730xl or 3100 capillary sequencer (Applied Biosystems, Carlsbad, California). The results were accessed via the LabLink server on the TCAG website, downloaded as .fsa format files, and viewed in the ABI PeakScanner version 2.0 program (Applied Biosystems Inc, Carlsbad, California).

The PeakScanner displays the profile of DNA fragments in a sample in a plot format and provides the sizes and absolute peak height of each fragment in a text format. The peak height of a PCR product is determined by the copy number of its template and the efficiency of PCR. By controlling the number of amplification cycles and PCR conditions optimally, the amplification reflected in the peak height can be linear to the copy number of its template DNA. Nevertheless, the absolute peak height can be further affected by many other factors, such as the variation of total DNA amount, amount of PCR product loaded in the analysis, etc, making the comparison between samples not very meaningful. A solution to this is to use internal control regions, which are known to be not subject to copy number variation. By calculating the ratio of peak heights between test regions and the control regions within the same sample, it limits the variation of this relative peak height to the variation of the relative copy number of the templates for the control and test regions, and makes it suitable for comparison between samples that are processed using the same protocol. A higher relative peak would indicate more copies, whereas, a smaller relative peak would indicate fewer copies. Since it is not easy to identify a sample with the "normal" copy number of the TRPM5 genes without using

another copy number detection method, the average relative peak height of a region was used within the sample cohort as the base line for detection of copy number gain and loss.

Analysis was performed by comparing the relative peak heights for the control regions (3 amplicons outside CNV regions)—3q, 11q, and Control1—to the regions within the CNV regions (5 amplicons): Marker1, TSPAN32, TRPM5_E22, TRPM5_E3, and KCNQ1. This was performed by using the following equation:

Proportion of peak height = Peak height of amplicon of interest / Average of control peak heights

The peak height proportions were then compared to one, two, and three standard deviations of the mean proportion for each site among all samples to identify potential copy number variations. Additionally, the average peak proportion for each site was compared for thermal tasters and thermal non-tasters.

SECTION III: RESULTS

III.1: Subjects' sensory ratings

A total of 60 participants were tested using prototypical tastants for sweet, sour, bitter, and umami, as well as astringency for mouth-feel. Maximum intensity ratings were recorded on gVAS paper scales (Appendix I for raw ratings), which were later normalized using a non-oral stimuli rating (brightness of the sun) from the scale acclimation process, by multiplying the intensity rating by the normalization factor. These normalized ratings were used for statistical analysis assessing the potential associations between the intensity ratings of these orosensory sensations (Appendix III).

$$\text{Normalization Factor} = \frac{\text{subjects brightness of the sun rating}}{\text{average brightness of the sun rating}}$$

PROP taster status was determined by each subject rating a 3.2mM solution of 6-*n*-propylthiouracil on a quasi-logarithmic gLMS paper scale, as this scale had been shown to provide good separation between taster groups (Bartoshuk et al., 2003). There were four different PROP ratings/classifications used in this study: non-normalized PROP taster status (PTS), non-normalized ratings (PROP), normalized PROP taster status (nPTS), and normalized ratings (nPROP) (Appendix IV). Data from each of these ratings was used to test the association between PROP taster status and each of the genotyped SNPs and to compare the effect of different ratings on the strength of the association (see section III.4).

Following normalization of PROP taster status, there were 27 subjects whose taster classification changed. Seven subjects who were PROP non-tasters were classified as PROP medium tasters after normalization, five PROP medium tasters were PROP

super tasters after normalization, and lastly 15 PROP super tasters were PROP medium tasters after normalization. There were no subjects who became non-tasters from tasters after normalization.

The last phenotypic data for this study was thermal taster status. There were 28 thermal non-tasters, 30 thermal tasters who experienced different tastes depending on the temperature cycle and the subject, and 8 non-classifiable subjects whose ratings were either too low (below "weak") or inconsistent between trials. This resulted in a sample size of 58 subjects for the thermal taster status analysis. The tastes experienced by the thermal tasters are listed in Table 7 and demonstrate a wide range in the tastes evoked by thermal stimulation.

Table 7: Tastes experienced by thermal tasters for heating and cooling trials.

Subject	TTS	Tastes Experienced	
		Warming	Cooling
141	TT	salty	salty, bitter
183	TT	bitter	
191	TT		sour
201	TT	salty	bitter
210	TT	salty, bitter	sweet, salty, bitter
221	TT	salty, sweet	sour
229	TT	salty, sour	salty, sour
338	TT		bitter
353	TT		bitter
379	TT	bitter	
413	TT	sweet, salty	
437	TT	salty, bitter	salty, sour
447	TT	sweet, salty	
499	TT		sour
513	TT	sweet	sour
526	TT	salty, bitter	
600	TT		bitter
639	TT	n/a	
680	TT	salty, bitter	salty, sour
885	TT	bitter	
894	TT		sour
899	TT		sour
970	TT	bitter	bitter, sour
1003	TT		sour
1004	TT	bitter	
1006	TT		sour
1012	TT		bitter, sour
1019	TT		salty
1020	TT	sweet	
1022	TT	sour	

While these individuals in Table 7 are all classified as thermal tasters, each individual exhibited different taste sensations either on heating or cooling of regions of the tongue and sometimes on both temperature cycles. It was proposed that perhaps there are different classifications of thermal tasters based on the temperature cycle in which the taste is experienced and by the tastes experienced as a result of the different taste

pathways. Therefore, thermal tasters were evaluated as a whole classification as well as by taste experienced and cycle experienced as the data demonstrates in Table 7.

III.2: Genotypes of SNPs for all genes studied

Non-synonymous coding single nucleotide polymorphisms (SNPs) were selected from dbSNP based the frequency within the population and the level of evolutionary conservation at the site of the protein involving the SNPs for each gene of interest. Consequently, the SNPs selected could result in changes to the function of the receptors and potentially the ability to bind to a tastant, thereby affecting the tasting capacity of the individual. These variations, such as those known in TAS2R38, could be responsible for different taster phenotypes such as PROP taster status, thermal taster status, or variations in the perceived intensities of other taste modalities and mouth-feel.

The genotypes of the selected SNPs were determined through Sanger sequencing of PCR products for each gene or region of the gene that contained the SNPs. The genotypes for each of the SNPs are presented in Tables 8–10. These genotypes were used for all of the statistical analyses to determine potential association between taster statuses and the genotypes. For statistical analysis, only the SNPs studied within TAS2R38, Gustin, and TRPM5 were analyzed. The other TAS2R genes that were genotyped were either sequenced for only a few individuals or there was little to no variation among those genotyped. The genotypes for the SNPs reported to be associated with PROP taster status (Cabras et al., 2012; Calņ et al., 2011; Duffy et al., 2004; Kim et al., 2003; Padiglia et al., 2010) are presented in Table 8, and these SNPs are rs713598, rs1726866, and rs10246939 in TAS2R38 and the Gustin SNP rs2274333.

Table 8: Genotypes for selected SNPs in TAS2R38 and Gustin.

Subject	rs713598	rs1726866	rs10246939	rs2274333
141	g/g	t/C	a/G	A/A
175	g/C	t/C	a/G	A/A
183	g/C	C/C	G/G	A/A
191	g/g	t/C	a/G	A/g
201	g/g	t/t	a/a	A/A
210	g/C	t/C	a/G	A/A
221	g/g	t/t	a/a	A/A
229	g/g	t/t	a/a	A/A
260	g/C	t/C	a/G	A/g
336	g/C	t/C	a/G	g/g
338	g/C	t/C	a/G	A/A
341	g/g	t/t	a/a	
379	g/g	t/t	a/a	g/g
400	g/C	t/C	G/G	A/g
413	g/C	t/C	a/G	A/g
420	g/g	t/t	a/a	A/g
437	g/C	C/C	G/G	A/A
446	g/C	C/C	G/G	A/A
447	g/g	t/t	a/a	A/A
464	C/C	C/C	G/G	
465	g/C	C/C	a/G	A/A
489	g/C	t/C	a/G	g/g
497	g/C	t/C	a/G	A/A
499	g/C	t/t	a/G	A/g
513	g/C	t/C	a/G	
526	g/C	t/C	a/G	A/A
538	g/C	C/C	G/G	A/A
559	g/C	t/C	a/G	A/A
573	g/g	t/t	a/a	
600	g/g	t/t	a/a	A/A
606	g/C	t/C	a/G	A/A
639	g/C	t/C	a/G	A/g
723	g/C	t/C	a/G	g/g
779	C/C	C/C	G/G	A/A
885	g/g	t/t	a/a	A/g
894	g/g	t/t	a/a	A/A

Subject	rs713598	rs1726866	rs10246939	rs2274333
899	C/C	C/C	G/G	A/A
948	C/C	C/C	G/c	A/A
970	g/C	t/C	a/G	
988	g/g	t/t	a/a	
994	C/C	C/C	G/G	A/A
1001	g/C	t/C	a/G	A/A
1002	g/C	t/C	a/G	A/g
1003	g/C	t/C	a/G	A/A
1004	g/g	C/C	G/G	A/A
1005	g/C	C/C	G/G	A/g
1006	g/g	t/C	a/G	g/g
1007	C/C	C/C	G/G	A/A
1008	g/C	t/C	a/G	A/A
1009	C/C	C/C	G/G	A/A
1012	g/g	t/t	a/a	g/g
1014	g/g	t/t	a/a	A/A
1015	g/C	t/C	a/G	A/A
1016	g/g	t/t	a/a	g/g
1018	g/g	t/t	a/a	A/g
1019	C/C	C/C	G/G	A/A
1020	C/C	C/C	G/G	
1022	g/C	C/C	G/G	A/A
1023	g/C	t/C	a/G	A/g
1024	g/C	t/C	a/G	A/g

*Upper case is the ancestral allele, lower case is the minor allele; empty cells indicate undetermined genotypes.

It is evident that the three TAS2R38 SNPs are typically one of two haplotypes: GTA/GTA (PAV/PAV) or CCG/CCG (AVI/AVI) and the heterozygous of the two haplotypes. This indicates that these three SNPs are inherited together, and recombination events are not likely to occur within the TAS2R38 gene resulting in the breaking of these haplotypes. The genotype data in Table 9 is for SNPs rs79526258, rs886277, rs3986599, rs34350821, rs34364959, rs111504104, and rs61741881, which are all found within TRPM5.

Table 9: Genotypes for TRPM5 SNPs.

Subject	rs79526258	rs886277	rs3986599	rs34350821	rs34364959	rs111504104	rs61741881
141					C/C		
201					C/t		
210					C/C		
221	C/C	t/t	t/t	C/C	C/C	G/G	C/C
229	C/C	t/t	C/C	C/C	C/C	G/G	C/C
338					C/C		
379					C/C		
420	C/C	t/t	t/t	C/C	C/C	G/G	C/C
437					C/t		
446	C/C				C/t	G/G	C/C
447	C/C				C/C	G/G	C/C
499	C/C	t/t	C/t	C/C	C/C	G/G	C/C
513					C/C		
526	C/C	t/t	C/C	C/C	C/C	G/G	C/C
538	C/C	t/t	C/t	a/a	C/t	G/G	C/C
559	C/C	t/t	C/C	C/C	C/t	G/G	C/C
600	a/C	C/C	C/C	C/C	C/C	G/G	C/C
680					C/C		
779	C/C	t/t	t/t	C/C	C/C	G/G	C/C
885					C/C		
894					C/C		
899					C/C		
948		t/t		C/C	C/C	G/G	C/C
970	C/C	t/t	t/t	C/C	C/C	G/G	C/C
994	C/C	C/C	C/C	C/C	C/C		C/C
1003	C/C	t/t	C/C	a/a	C/t	G/G	C/C
1004					C/C		
1006	C/C	t/t	C/t	C/C	C/t	G/G	C/C
1008	C/C	t/t	C/C	C/C	C/C	G/G	C/C
1012	C/C	t/t	t/t	C/C	C/C	G/G	C/C
1014	C/C	t/t	t/t	C/C	C/C		C/C
1016	C/C	t/t	C/C	C/C	C/C	G/G	C/C
1020					C/C		
1022					C/C		

*Upper case is the ancestral allele, lower case is the minor allele; empty cells indicate undetermined genotypes.

It is clear from the genotypes of the TRPM5 SNPs—rs111504104, rs61741881, and rs79526258—that variations at these SNPs sites are not present in this population because all of the subjects have the same genotype for these SNPs with the exception of

one subject for rs79526258, who is heterozygous. Therefore, these SNPs were excluded from analysis along with rs886277 and rs34350821, which have only two subjects with a genotype different from the rest of the studied population. This leaves two SNPs within TRPM5 to analyze, rs34364959 and rs3986599.

In addition, SNPs within a few other TAS2R genes were genotyped for a small set of subjects based on their PROP taster status. Of the 13 SNPs selected from the TAS2R genes, only some of the SNPs for TAS2R16 were sequenced based on the preliminary screening. Most of the SNPs in the TAS2R genes resulted in a lack of variation among the subjects; thus they were excluded from the study. The genotypes for the TAS2R16 SNPs sequenced are in Table 10.

Table 10: Genotypes for TAS2R16 SNPs.

Subject	PTS	rs846664	rs978739	rs860170
183	PNT	t/t	A/g	A/g
210	PNT	t/t	g/g	A/A
446	PNT	t/t	A/g	A/g
526	PST	t/t	g/g	A/A
538	PST		A/g	
559	PST	t/t	g/g	
606	PNT	t/t	g/g	A/A
639	PST	t/t	g/g	A/g
970	PST		g/g	
1003	PST	t/t	g/g	A/A
1005	PST	t/t	g/g	A/g
1006	PST		g/g	
1008	PST	t/t	g/g	g/g
1014	PNT	t/t	g/g	A/A
1015	PST	t/t	g/g	A/A
1016	PNT	G/G		
1023	PST	t/t	g/g	A/A

*Upper case is the ancestral allele, lower case is the minor allele; empty cells indicate undetermined genotypes.

Upon manual examination of the genotypes in Table 10 and the phenotype data, particularly for PROP taster status and thermal taster status, it was concluded that there

was a lack of variation in the subjects. There was no difference between the PROP taster status groups and thermal taster status groups for these SNPs. Therefore, these SNPs were excluded from the statistical analysis and no further genotyping was performed for them.

III.3: Association studies between genotypes and orosensory sensations

Each genotyped SNP as well as combined SNPs for individual genes were analyzed for potential associations with the following phenotypic characteristics: normalized astringency, bitterness, sourness, sweetness, and umami ratings as determined using prototypical tastants and an astringent. Associations were assessed using Pearson's correlation coefficient (data not shown) and regression analysis in SAS 9.2. Each of the studied attributes (astringency, bitterness, sourness, sweetness, and umami) was assessed statistically for associations with the SNPs within TAS2R38, TRPM5, and Gustin. However, only those associations that were statistically significant are shown in Table 11 (non-significant results are in Appendix II).

Table 11: A summary list of significant associations between SNPs and an oral sensation.

Attribute	SNP(s)	F-value	R-squared	p-value
Astringency	rs713598 and rs1726866	3.22	0.10	0.0475*
Astringency	rs713598 and rs10246939	3.69	0.12	0.0314*
Bitterness	rs713598 and rs1726866	6.19	0.18	0.0038**
Bitterness	rs713598 and rs10246939	6.19	0.18	0.0038**
Sourness	rs713598 and rs10246939	3.38	0.11	0.0414*
Sweetness	rs713598 and rs1726866	5.96	0.18	0.0046**
Sweetness	rs713598 and rs10246939	5.01	0.15	0.01**
Sweetness	rs713598, rs1726866 & rs10246939	4.02	0.18	0.0119**

* Statistical significance is set at $p \leq 0.05$, **statistical significance following Bonferroni correction.

Interestingly, of all the SNPs studied in TAS2R38, TRPM5, and Gustin, only the SNPs within TAS2R38 known to be associated with PROP taster status were significantly associated with astringency, bitterness, sourness and sweetness ratings. Furthermore, the significant associations were seen only when two or more SNPs were combined, not each SNP individually (Table 11).

III.4: PROP taster status and SNP associations

Genotyping of PROP super tasters, medium tasters, and non-tasters (PST, PMT, and PNT respectively) was performed for TAS2R38, Gustin, TRPM5, and other TAS2R genes. Due to previous research indicating its significant contribution (Duffy et al., 2004; Hayes et al., 2008), TAS2R38 was first analyzed to determine the degree to which it contributes to PROP taster status determination in the sample cohorts.

Since there are many ways to evaluate PROP taster status, each of the following attributes were evaluated for associations with the TAS2R38 SNPs to determine which PROP phenotype classification exhibits the strongest relationship: PROP taster status (PTS), PROP ratings, normalized PTS (nPTS), and normalized PROP ratings (nPROP rating) (Tables 12–14).

Table 12: Pearson's correlation coefficient for the TAS2R38 SNPs and PROP phenotypes.

Classification of PROP tested	SNP	Correlation Coefficient	p-value
PTS	rs713598	0.58	<0.0001**
PTS	rs1726866	0.60	<0.0001**
PTS	rs10246939	0.62	<0.0001**
PROP rating	rs713598	0.52	<0.0001**
PROP rating	rs1726866	0.53	<0.0001**
PROP rating	rs10246939	0.52	<0.0001**
nPTS	rs713598	0.39	0.0042**
nPTS	rs1726866	0.38	0.0057**
nPTS	rs10246939	0.43	0.0016**
nPROP rating	rs713598	0.23	0.0976*
nPROP rating	rs1726866	0.43	0.0015**
nPROP rating	rs10246939	0.45	0.001**

* Statistical significance is set at $p \leq 0.05$, **statistical significance following Bonferroni correction.

From the results of the Pearson's correlation, the strongest association for each SNP with the phenotype was for PTS. The weakest association was with the normalized PROP ratings, in which rs712598 was not significantly associated with the ratings. Additionally, regression analysis was performed for the SNPs with the same combinations of PROP phenotypes (Table 13 for PTS and PROP ratings and Table 14 for the nPTS and nPROP ratings).

Table 13: Regression analysis for the TAS2R38 SNPs, PTS and PROP ratings.

PROP status	SNP(s)	F-value	R-Squared	p-value
PTS	rs713598	25.87	0.34	<0.0001**
PTS	rs1726866	28.53	0.36	<0.0001**
PTS	rs10246939	31.72	0.38	<0.0001**
PTS	rs713598 and rs1726866	16.17	0.39	<0.0001**
PTS	rs713598 and rs10246939	17.49	0.41	<0.0001**
PTS	rs1726866 and rs10246939	15.77	0.39	<0.0001**
PTS	rs713598, rs1726866 & rs10246939	11.43	0.41	<0.0001**
PROP rating	rs713598	18.85	0.27	<0.0001**
PROP rating	rs1726866	19.58	0.28	<0.0001**
PROP rating	rs10246939	13.98	0.27	<0.0001**
PROP rating	rs713598 and rs1726866	11.16	0.31	<0.0001**
PROP rating	rs713598 and rs10246939	11.08	0.31	<0.0001**
PROP rating	rs1726866 and rs10246939	9.91	0.28	0.0002**
PROP rating	rs713598, rs1726866 & rs10246939	7.38	0.31	0.0004**

* Statistical significance is set at $p \leq 0.05$, **statistical significance following Bonferroni correction.

Table 14: Regression analysis for the TAS2R38 SNPs nPTS and nPROP ratings.

PROP status	SNP(s)	F-value	R-Squared	p-value
nPTS	rs713598	9.01	0.16	0.0042**
nPTS	rs1726866	8.36	0.15	0.0057**
nPTS	rs10246939	11.18	0.19	0.0016**
nPTS	rs713598 & rs1726866	4.93	0.17	0.0112**
nPTS	rs713598 & rs10246939	5.88	0.20	0.0052**
nPTS	rs1726866 & rs10246939	5.58	0.19	0.0066**
nPTS	rs713598, rs1726866 & rs10246939	4.02	0.20	0.0126**
nPROP rating	rs713598	2.85	0.06	0.0976
nPROP rating	rs1726866	11.38	0.19	0.0015**
nPROP rating	rs10246939	12.35	0.20	0.001**
nPROP rating	rs713598 & rs1726866	6.67	0.22	0.0028**
nPROP rating	rs713598 & rs10246939	8.64	0.26	0.0006**
nPROP rating	rs1726866 & rs10246939	6.07	0.20	0.0045**
nPROP rating	rs713598, rs1726866 & rs10246939	5.64	0.26	0.0022**

* Statistical significance is set at $p \leq 0.05$, **statistical significance following Bonferroni correction.

From the results in Tables 13 and 14, it is evident that the strongest association is when the non-normalized PTS is used. Therefore, for the remainder of the statistical analysis of PROP taster status and the other genes' SNPs (Gustin and TRPM5), the non-normalized PTS classification was used.

Since the TAS2R38 SNPs are responsible for only approximately 41% of PROP taster status determination (Table 13), one would expect to have some subjects in which the phenotype and genotype do not coincide. In this study, there were four subjects who were PROP non-tasters with a taster genotype (two of each PAV/AVI and PAV/AAV) and 14 subjects who were PROP super tasters with the medium tasting genotype (PAV/AVI) (Table 8).

The next gene studied was TRPM5, which is a temperature-sensitive ion channel involved in the bitter taste transduction pathway (Zhang et al., 2003). Only the two SNPs previously assessed for each orosensory sensation intensity rating were analyzed for PTS (Table 15), with the rest of the SNPs not considered due to a lack of variation among subjects and incomplete genotyping of the subjects. The TRPM5 SNPs rs3986599 and rs34364959 were not significantly associated with PROP taster status based on both the Pearson's correlation (data not shown) and regression analysis.

Table 15: Regression analysis for the TRPM5 SNPs and PROP taster status.

SNP(s)	F-value	R-Squared	p-value
rs3986599	0.63	0.04	0.4390
rs34364959	1.43	0.04	0.2400
rs3986599 & rs34364959	3.22	0.32	0.0710

The last gene to be analyzed for associations with PROP taster status was Gustin. The association of the Gustin SNP with PROP tasting was tested using a few methods of coding. The methods of coding for genotype were the inclusion of the heterozygous genotype (A/G) or testing only for the presence of the minor allele (A). The coding methods for phenotype were the inclusion of the PMT phenotype (trimodal classification) or the combination of PMTs and PSTs to make a "taster" phenotype (binomial classification) (Table 16).

Table 16: Regression analysis for the Gustin SNP rs2274333 and PROP taster status.

	F-value	R Squared	p-value
Trimodal PTS	0.05	0.00	0.8184
Binomial PTS	0.02	0.00	0.8918
Trimodal PTS and Presence of Minor Allele	1.10	0.02	0.2999
Binomial PTS and Presence of Minor Allele	0.42	0.01	0.5211

Unexpectedly, none of the coding combinations for the Gustin genotype or PROP taster status resulted in a significant association between the Gustin genotype and PROP taster status for both the Pearson's correlation (data not shown) and regression analysis. This was not expected because as stated previously, the SNP rs2274333 in Gustin was previously reported to be significantly associated with PROP taster status (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010). Furthermore, upon examination of the genotypes, there were 14 of the PROP non-tasters with the known taster genotype (eight with A/A and six with A/G) for the Gustin SNP instead of the expected non-taster genotype (G/G), suggesting the need of re-evaluating of the association of Gustin with PTS. Therefore, even though many SNPs were genotyped and analyzed, only those three SNPs within TAS2R38 were found to be significantly associated with PROP taster status. Those three SNPs, however, can explain only 41% of PROP taster status, indicating there are still other factors involved in PROP taster status determination.

III.5: Thermal taster status and SNP associations

The mechanism behind thermal taster status is not currently known; therefore, genotyping of genes of interest, particularly TRPM5, could provide insight into a currently unexplained phenomenon. First, it is known that thermal taster status is not associated with PROP taster status phenotypically (Bajec & Pickering, 2008). Therefore,

thermal taster status was analyzed genetically with TAS2R38 to confirm this lack of association. The same analyses as for the orosensory sensations were performed for thermal taster status and the TAS2R38 SNPs (Table 17). It is clear that there is no association between the TAS2R38 SNPs and thermal taster status as was hypothesized.

Table 17: Regression analysis for the TAS2R38 SNPs and thermal taster status.

SNP(s)	F-value	R-Squared	p-value
rs713598	2.32	0.04	0.1336
rs1726866	0.77	0.01	0.3834
rs10246939	0.29	0.01	0.5924
rs713598 and rs1726866	1.24	0.05	0.2970
rs713598 and rs10246939	1.60	0.06	0.2117
rs1726866 and rs10246939	0.69	0.03	0.5078
rs713598, rs1726866 & rs10246939	1.18	0.07	0.3262

Next, TRPM5, which has been proposed to be associated with thermal taster status (Talavera et al., 2005), was analyzed for a potential association. As with the other phenotypes, only two SNPs were assessed statistically since variation was visibly evident in the genotypes of these SNPs (Table 18).

Table 18: Regression analysis for the TRPM5 SNPs and thermal taster status.

SNP(s)	F-value	R-Squared	p-value
rs3986599	0.00	0.00	0.9766
rs34364959	0.96	0.03	0.3350
rs3986599 & rs34364959	0.01	0.00	0.9925

It is evident that thermal taster status was not significantly associated with the TRPM5 SNPs studied based on either the Pearson's correlation (data not shown) or the regression analysis. However, TRPM5 is heat-sensitive and known to be part of only bitter, sweet, and umami taste transduction, whereas thermal taste was assessed with cooling and heating cycles combined; this could be the reason a lack of association was found. Consequently, these SNPs were assessed manually for thermal tasters with

sensitivity to the heat cycle and experienced tastes of bitter and sweet to determine if there was a potential association. For both SNPs, variation in the genotypes was not observed upon this manual examination to show any association with thermal taster status. This does not coincide with previous work, which had suggested TRPM5 as a potential candidate gene for thermal taste (Talavera et al., 2005). Thermal taster status was also analyzed for an association with the Gustin SNP rs2274333, and it was shown to be not associated as expected (Appendix II).

III.6: Thermal taster status and TRPM5 copy number variations

Since there were no significant associations between thermal taster status and any of the SNPs genotyped, TRPM5 was assessed for copy number variations and a potential association with thermal taster status. The entire TRPM5 gene is known to be subjected to copy number variations; therefore, it was a good candidate for potential copy number variations and associations with phenotypes such as thermal taster status. Copy numbers were assessed using an upQMPSF (quantitative multiplex PCR of short fluorescent fragments) procedure with capillary electrophoresis analysis. The results of the electrophoresis were assessed for relative peak heights against controls on additional chromosomes. An example of the capillary electrophoresis results can be found in Figure 6.

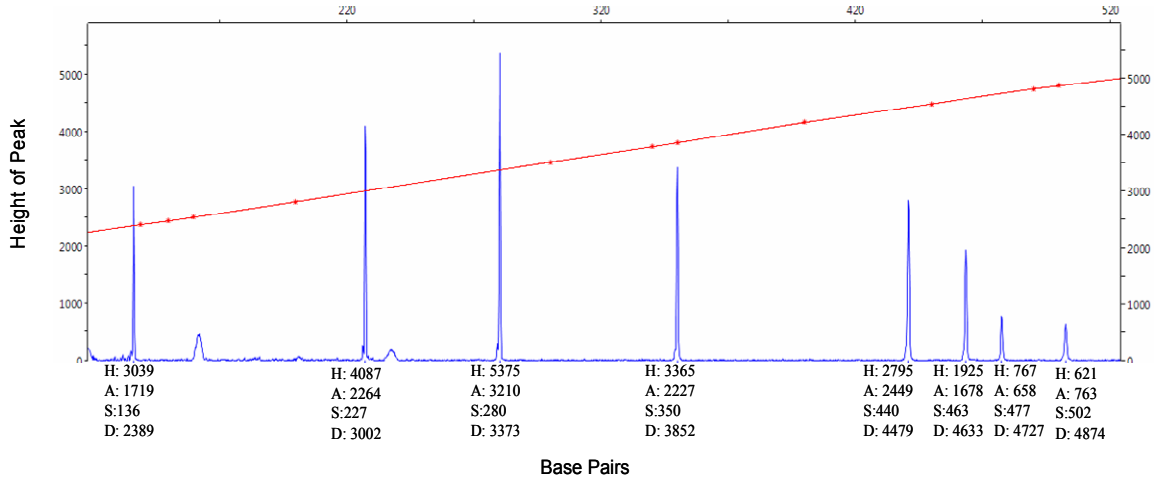

Figure 6: An example of the upQMPSF profile by capillary electrophoresis. Shown in the example is the upQMPSF profile for sample 1004 viewed in the Applied Biosciences Peak Scanner 2.0 on base pairs view. X-axis legend H: height of peak, A: area contained within the peak, S: base pair size and D: scan number. The red line is the sizing standard curve used to determine the base pair sizes of the detected products. Peaks from left to right: 3q, Control 1, 11q, Marker 1, TSPAN32, TRPM5_E3, KCNQ1, and TRPM5_E22).

Each sample's image output was measured for peak heights and tabulated (Appendix V). There were six controls within each reaction: 3q, 11q, Control1, TSPAN32, Marker1, and KCNQ1. After assessing the absolute peak heights, averages of the control peaks (3q, 11q and Control1) were used to determine relative peak heights for the test regions (Table 19).

Table 19: Average control peak heights and the relative peak height of test region.

Subject	TTS & Taste*	Average	TSPAN32 -0.7	TRPM5_E3 -0.46	TRPM5_E22 -0.12	Marker1 -0.95	KCNQ1 -0.35
336	TnT	3228	0.74	0.35	0.08	0.79	0.33
341	TnT	397	0.54	0.25	0.08	0.72	0.49
400	TnT	692	0.73	0.56	0.15	1.08	0.46
420	TnT	408	0.48	0.29	0.09	0.72	0.3
446	TnT	663	0.87	0.49	0.11	0.96	0.3
464	TnT	1026	0.88	0.68	0.16	1.17	0.39
465	TnT	1103	0.94	0.64	0.14	1.1	0.33
489	TnT	1144	0.97	0.82	0.21	1.15	0.41
538	TnT	1061	0.7	0.42	0.1	0.98	0.39
559	TnT	807	0.75	0.57	0.17	1.14	0.42
573	TnT	1751	0.59	0.46	0.13	1.01	0.36
723	TnT	1505	0.8	0.54	0.13	0.98	0.31
779	TnT	1338	0.52	0.32	0.09	0.9	0.38
948	TnT	4199	0.66	0.5	0.13	1.11	0.29
994	TnT	1162	0.62	0.44	0.11	0.97	0.31
1005	TnT	2050	0.76	0.42	0.12	0.87	0.22
1008	TnT	2407	0.79	0.47	0.13	0.85	0.2
1014	TnT	2581	0.71	0.41	0.12	0.66	0.19
1016	TnT	2579	0.85	0.62	0.18	1.01	0.27
183	TT b	594	0.66	0.45	0.12	0.89	0.36
338	TT b	1053	0.69	0.5	0.13	1.05	0.49
379	TT b	319	0.62	0.36	0.15	0.88	0.45
600	TT b	1235	0.69	0.45	0.09	1.02	0.34

Subject	TTS & Taste*	Average	TSPAN32	TRPM5_E3	TRPM5_E22	Marker1	KCNQ1
			-0.7	-0.46	-0.12	-0.95	-0.35
885	TT b	982	0.75	0.49	0.1	1.08	0.55
1004	TT b	4167	0.67	0.46	0.15	0.81	0.18
141	TT b/sa	1325	0.42	0.36	0.02	1.01	0.12
526	TT b/sa	578	0.75	0.47	0.15	1.14	0.41
437	TT b/sa/so	612	0.46	0.17	0.06	0.59	0.28
970	TT b/so	1007	0.56	0.26	0.08	0.86	0.41
1012	TT b/so	1616	0.81	0.63	0.2	1.02	0.23
639	TT NA	265	0.56	0.5	NA	0.81	0.26
229	TT sa/so	970	0.64	0.51	0.12	1.11	0.46
499	TT so	926	0.79	0.63	0.16	1.16	0.46
894	TT so	298	0.63	0.23	0.11	0.79	0.42
899	TT so	1704	0.78	0.41	0.09	0.94	0.3
1003	TT so	3213	0.59	0.33	0.1	0.65	0.12
1006	TT so	3038	0.59	0.29	0.07	0.69	0.17
1022	TT so	2923	0.76	0.46	0.1	0.87	0.22
413	TT sw	723	0.59	0.37	0.1	1.02	0.34
447	TT sw	1709	0.89	0.65	0.13	1.21	0.54
1020	TT sw	1933	1.03	0.86	0.15	1.23	0.41
201	TT sw/b/sa	314	0.65	0.39	0.14	0.86	0.46
210	TT sw/b/sa	941	0.54	0.42	0.13	0.94	0.41
221	TT sw/sa/so	232	0.58	0.48	0.2	0.8	0.37
513	TT sw/so	323	0.74	0.48	0.14	1.06	0.53

*Tastes experienced on heating or cooling trials; bitter (b), sweet (sw), sour (so), salty (sa).

The relative height of an individual test region was then compared to the average relative heights of this region among all samples to detect any significant deviation from the average, as an indication of copy number gain or loss. All subjects' peak heights were within three standard deviations of the mean proportions, with five subjects having regions showing relative peak heights outside of two standard deviations (highlighted values in Table 20, showing only samples with one or more regions with significant relative peak height deviation), suggesting possible copy number gain (shown in red) or loss (shown in blue). The regions showing possible copy number variation include TRPM5 regions, TRPM5_E5 and TRPM5_E22. However, since the apparent copy number gain and loss are both seen in TT subjects, it is evident that there is no association between the copy number regions studied here and thermal taster status.

Table 20: Subjects and the relative peak heights for TRPM5 outside two standard deviations of the average in the header.

Subject	TTS & Taste	TSPAN32 (0.70[#])	TRPM5_E3 (0.46)	TRPM5_E22 (0.12)	Marker1 (0.95)	KCNQ1 (0.35)
489	TnT	0.97	0.82	0.21	1.15	0.41
141	TT b/sa	0.42	0.36	0.02	1.01	0.12
437	TT b/sa/so	0.46	0.17	0.06	0.59	0.28
1003	TT so	0.59	0.33	0.10	0.65	0.12
1020	TT sw	1.03	0.86	0.15	1.23	0.41

* Red indicates gain, blue indicates loss. [#]Average peak height among all samples.

Lastly, to confirm a lack of association, the averages of peak proportions for TTs and TnTs were determined (Table 21). Since these average peak proportions in Table 21 are quite similar between the thermal tasters and thermal non-tasters, it is unlikely the copy number variations observed within TRPM5 are associated with thermal taste. This result coincides with the SNP results for TRPM5, which showed no significant

association between the genotypes of the SNPs and thermal taster status. Consequently, further investigation into other contributing factors and genes is necessary to determine the cause of thermal taste variation.

Table 21: Average relative peak heights for the copy number regions for TTs and TnTs.

TTS	Number of Subjects	TSPAN32	TRPM5_E3	TRPM5_E22	Marker1	KCNQ1
TT	26	0.67	0.45	0.12	0.94	0.36
TnT	19	0.73	0.49	0.13	0.96	0.33

III.7: Summary of results

In summary, PROP taster status was shown to be significantly associated with the three SNPs within TAS2R38 as was reported by earlier studies. The greatest association was the non-normalized PROP taster status with all three TAS2R38 SNPs having an R-squared value of 0.4118. PROP taster status was, however, not associated with either of the TRPM5 SNPs studied or with the Gustin SNP rs2274333, despite the previous reported association with PROP taster status for Gustin.

The orosensory sensation ratings assessed for associations with the SNPs within TAS2R38, TRPM5, and Gustin showed significant associations with TAS2R38 when more than one SNP was considered in the association analysis. This is likely due to the association between increased sensitivity to orosensory sensations and PROP taster status, as TAS2R38 is a component of PROP taster status determination.

Lastly, thermal taster status was not significantly associated with any of the SNPs studied. As with the SNPs, there were no associations found with the copy number variations within TRPM5 or the surrounding area and thermal taster status, thus leaving the genetic basis of thermal tasting unsolved.

SECTION VI: DISCUSSIONS

There are many known taste receptors specific to each taste. Sweet, umami, and bitter compounds are detected through 7-transmembrane receptors known as TAS1R and TAS2R (Chandrashekar et al., 2000; Jiang et al., 2004; Li et al., 2002; Nelson et al., 2001; Xu et al., 2004). Sour and salty tastes are detected through ion channels for hydrogen and sodium ions respectively (Chaudhari & Roper, 2010). This study focused on the genetic contributions of TAS2R38, Gustin, and TRPM5 to the taste phenotypes: PROP taster status, thermal taster status, and orosensory sensation ratings. Sixty-one participants were evaluated for their PROP and thermal taster status and were later genotyped for single nucleotide polymorphisms for these three genes, as well as copy number variations for TRPM5. The objectives of this study were to determine what genes and variations contribute to PROP and thermal taster statuses, and how intensity ratings for basic tastes and astringency are associated with these genetic contributions.

The genetic basis of PROP taster status

One of the purposes of this study was to identify potential associations between the genotypes of single nucleotide polymorphisms (SNPs) and PROP taster status in TAS2R38, Gustin, and TRPM5. The factors behind PROP taster status determination are vital to understanding how PTS is related to the phenotypic attributes discovered to be associated with it. Some of these phenotypic attributes include increased perceived intensity of orosensory sensations, alcohol consumption, BMI, fat perception, and food preference (Bajec & Pickering, 2008; Duffy et al., 2004; Duffy, 2007; Hayes et al., 2011; Tepper & Nurse, 1997; Tepper & Ullrich, 2002; Tepper, 2008). Significant research also

exists to contradict the associations among PTS and BMI, food preference, and fat perception (Drewnowski et al., 2007; Duffy & Bartoshuk, 2000; Yackinous & Guinard 2001).

As with previous research, PROP taster status was found to be significantly associated with the three TAS2R38 SNPs: rs713598, rs1726866, and rs10246939 (Duffy et al., 2004; Kim et al., 2003). The TAS2R38 SNPs occur in three common haplotypes: PAV/PAV, PAV/AVI, and AVI/AVI, resulting in PROP super tasters, medium tasters, and non-tasters respectively (Duffy et al., 2004). Based on evolutionary conservation assessed through the multiple protein sequence alignments performed, the AVI/AVI genotype for PROP non-tasters is relatively new, occurring only in humans. This is likely the result of the decreased need for toxin avoidance mechanisms such as the PAV/PAV genotype of TAS2R38 typically found in other species based on dietary habits and the association between bitter taste and toxins (Meyerhof et al., 2011).

Following genotyping and statistical analysis, the strongest association for TAS2R38 was with the non-normalized PROP taster status and all three TAS2R38 SNPs. When analyzed together, the SNPs resulted in an R-squared value of 0.4118, indicating that the SNPs can explain 41% of PROP taster status determination. Consequently, there is still approximately 59% of the variation in PROP taster status that requires explanation. Caln et al. (2011) found that the TAS2R38 genotype could differentiate between only the PROP tasters and non-tasters and not between the PROP medium and super tasters. This finding would concur with the association found in this study. Additionally, Hayes et al. (2008) found that TAS2R38 could differentiate between tasters and non-tasters of PROP with the presence of the PAV genotype (heterozygous or homozygous), but it was not

capable of separating the super and medium tasters. In contrast, when suprathreshold concentrations (3.2mM) of PROP—the same concentration used for this study—were assessed for perception in PROP non-tasters with AVI/AVI TAS2R38 genotypes, the study's subjects rated PROP with greater bitterness intensity (Hayes et al., 2008) than the non-suprathreshold concentrations of PROP.

Since the TAS2R38 SNPs are responsible for only approximately 41% of PROP taster status determination, one would expect to have some subjects in which the phenotype and genotype do not coincide. As outlined previously, there were four subjects who were PROP non-tasters with a taster genotype (two of each PAV/AVI and PAV/AAV) and 14 subjects who were PROP super tasters with the medium tasting genotype (PAV/AVI). The PROP non-tasters with the tasting genotypes were of particular interest because they should have been able to taste PROP given their genotypes. Therefore, further analysis was performed to determine other potential reasons for their non-taster phenotypes. These subjects Gustin SNP rs2274333 genotypes were examined for potential associations with PROP taster status. For example, a non-taster genotype (G/G) would account for their non-tasting of PROP. However, all four of these subjects were A/A (the PROP taster genotype), thereby leaving the question of why these subjects were non-tasters. Upon normalization, one of these subjects rated the bitterness of PROP close to the non-taster cut-off (<10.9mm). This resulted in the subject becoming a PROP medium taster after normalization, leaving only three unexplained subjects. Other genes of interest were also genotyped for potential explanations; however, of all of the other SNPs genotyped (in the genes: TAS2R4, TAS2R13, TAS2R16, TAS2R19, and TAS2R44), none of the variations were consistent

among these individuals, thus leaving these subjects' PROP taster status still unexplained. Lastly, the ethnicity of these subjects was investigated to determine if they were of the same ethnic background. However, all four subjects listed themselves as Caucasian and born in North America as with the majority of the population studied. One possible explanation for these subjects could be their fungiform papillae counts, as it is known to be associated with PROP taster status (Hayes et al., 2008), but this data was not available for these subjects. After ruling out all possible sources of data available, the mystery of these PROP non-tasters with taster genotypes remains unsolved, leading to the question of what other factors are contributing to PROP taster status. Could there be other genes involved, or could there be variations in the neural transmission pathway?

As for the 14 PROP super tasters with medium taster genotypes, half of these subjects can be explained by their Gustin genotypes because they were A/A, which is reported to be associated with PROP super tasting (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010). The other seven subjects were A/G for the Gustin SNP; therefore, they would still be expected to be PROP medium tasters. Similar to this result, Cabras et al. (2012) demonstrated that only 69% of PROP super tasters studied had the PAV/PAV TAS2R38 genotype; thus subjects who are PROP super tasters with other genotypes seem to be quite common, suggesting the involvement of other genes.

This indicates, as stated previously, that there is more to PROP taster status determination than the TAS2R38, and other receptors are likely to be involved in the detection of suprathreshold PROP solutions (Hayes et al., 2008). It is evident that TAS2R38 not being able to explain all of PROP taster status determination coincides with previous findings and leads to the question of what else is contributing to PROP

taster status. Possible other factors contributing to PROP taster status could be the Gustin genotype (which will be discussed later), fungiform papillae counts, and other genes such as TRPM5 or other TAS2R receptors.

Fungiform papillae counts are known to be associated with PROP taster status (Bartoshuk et al., 1994; Essick et al., 2003; Hayes et al., 2008; Tepper & Nurse, 1997). The greater the density of the papillae, the greater the intensity of PROP perceived; however, this is found only in PROP tasters because PROP non-tasters lack a functional component of the PROP tasting pathway (Delwiche et al., 2001), such as the AVI/AVI TAS2R38 genotype. If included in this study, the papillae counts could have provided valuable insight into the association studies performed for PROP taster status.

Since papillae counts were not collected for the subjects tested, this proposed mechanism could not be assessed in this study; however, the Gustin SNP rs2274333 was genotyped for these individuals. Unexpectedly, it was found that PROP taster status was not associated with the Gustin SNP rs2274333 in this study, although an association as was found previously (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010). Upon further examination of the data, there were 14 PROP non-tasters with the "taster" genotype (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010). This finding may be the cause of the lack of association between Gustin and PROP taster status found through this research. This hurdle in genotyping analysis may have been overcome by having a larger sample size (which in this study was limited by financial and time constraints) in which there would be more PROP non-tasters with the non-taster genotype than the taster genotype.

One possible consideration is that all three previous studies on PROP taster status and the Gustin associations were performed solely on an Italian population (Caucasian) (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010). This study included subjects of other ethnicities (seven subjects) and even those who were defined as Caucasian were likely not all of Italian descent. This variability in ethnicity can significantly impact genotyping studies because different ethnicities are known to have different genotypes. Therefore, the Gustin SNP rs2274333 may be associated only with PROP taster status in a subset of the population—in this case, those of Italian ancestry—although more research is needed to confirm or deny this point.

Lastly, SNPs within TRPM5 were genotyped and analyzed for associations with PROP taster status. TRPM5 was suspected to be potentially associated with PROP taster status since it is part of the bitter taste transduction pathway. TRPM5 is stimulated by signals from secondary messengers or changes in the voltage of the cell as a result of signaling from other taste receptors (Liman, 2007). Therefore, if variations in the TRPM5 gene result in changes in the function of the protein, as non-synonymous SNPs could, taste transduction would be affected downstream of the TAS2R channels for bitter taste and potentially impact PROP taster status. Two SNPs—rs34364959 and rs3986599—were studied for potential associations with PROP taster status. However, neither of the SNPs was significantly associated with PROP taster status, again leaving the question of what else contributes to PROP taster status.

Therefore, of all the associations studied for PROP taster status, only the three SNPs within TAS2R38 were confirmed to be associated with PTS. However, this

association can only explain 41% of PROP taster status determination, therefore further research is needed into the mechanism behind PROP taster status.

Association between gene genotypes and orosensory sensations

Since PROP taster status and thermal taster status are known to be associated with increased perceived intensity to many oral tastants and mouth-feel, the five basic tastes and astringency ratings were assessed for potential associations with the genes studied. Interestingly, some of the attributes—astringency, as well as sweet, bitter, and sour taste—were significantly associated with the TAS2R38 genotypes, thus confirming the relationship between PROP taster status and perceived intensity ratings found previously (Bajec & Pickering, 2008).

One possible explanation for the significant associations between sweet, bitter, and sour taste could be the interaction between papillae count and increased taste sensitivity (Miller & Reedy, 1990) because PROP taster status is known to be associated with papillae density (Bartoshuk et al., 1994; Essick et al., 2003; Hayes et al., 2008; Tepper & Nurse, 1997). One way to confirm this possible explanation would be to determine the papillae counts for these subjects and then analyze those counts against their normalized orosensory sensation intensity ratings. The increased density of fungiform papillae would account for greater sensitivity to orosensory sensations because of the greater density of receptors, which are found within the fungiform papillae. This would allow for greater detection of a tastant (Bartoshuk et al., 1994).

However, the SNPs within TAS2R38 were significantly associated with the orosensory sensations only when the SNPs were analyzed together. Additionally, upon

using the Bonferroni correction, the significant associations between the SNPs, bitter and sour taste were no longer significant. Therefore, the significant associations found are likely the result of the interaction between the haplotypes of the SNPs rather than interactions between the intensity ratings and the SNPs, especially since the R-squared values for all of the associations are under 0.2, indicating weak associations. On the other hand, another explanation for the significant associations between the basic tastes (bitter, sour, and sweet) and astringency could be the increased perceived intensity to other tastes and mouth-feel in PROP super tasters (Duffy et al., 2004). PROP taster status is known to be associated with the increased perceived intensity of astringency as well as sweet, bitter, sour, and salty tastes (Bajec & Pickering, 2008; Gent & Bartoshuk, 1983; Lucchina et al., 1998; Prescott et al., 2004). Therefore, these significant associations are likely the result of the relationship between PROP taster status, TAS2R38, and increased intensity ratings for some basic tastes and astringency found by PROP tasters. This study supports previous phenotypic findings between PROP taster status and orosensory intensity ratings.

Genetic basis of thermal taste

Along with the other phenotypes studied, thermal taster status was also analyzed for potential associations with TAS2R38, Gustin, and TRPM5. Thermal taster status and PROP taster status are both known to be associated with increased intensity ratings for astringency, metallic taste, sweet, bitter, and salty taste, but no phenotypic association has been found between these two taster statuses (Bajec & Pickering, 2008). Hence, genotyping of TAS2R38 and the analyses for associations with thermal taster status was

necessary to confirm the lack of association between PROP and thermal taster status genetically. As expected, the TAS2R38 SNPs, rs713598, rs1726866, and rs10246939—known to be associated with PROP taster status (Duffy et al., 2004; Kim et al., 2003)—were not significantly associated with thermal taster status. This confirms previous findings of a lack of association between PROP and thermal taster status phenotypically with genetic confirmation.

Next, Gustin was assessed for a potential association with thermal taster status as with the other attributes studied. As with TAS2R38, the Gustin SNP rs2274333 was not expected to be associated with thermal taster status due to its reported association with PROP taster status (Cabras et al., 2012; Calņ et al., 2011; Padiglia et al., 2010), and the results from this study confirm this. Since both TAS2R38 and Gustin were not associated with thermal taster status, it can be concluded that PROP and thermal taster status are not genetically associated through these two genes.

To continue searching for a gene related to thermal taste, TRPM5 was studied for a potential association with both SNPs and CNVs. It was found that the two SNPs studied—rs34364959 and rs3986599—were not significantly associated with thermal taster status. Additionally, manual examination of TTs with sensitivity to the heat cycle and experienced tastes of bitter and sweet was performed to determine if there was a potential association. For both SNPs, variation in the genotypes was not observed upon this manual examination to show any association with thermal taster status.

As with the SNPs, CNVs were not found to be associated with TTS. In this population no consistent pattern of loss or gain in relative peak heights was observed. There were five subjects who displayed potential copy number variations within the

regions studied. However, these five subjects included both thermal tasters and thermal non-tasters. Four of these five subjects had relative peak heights for the two exons (TRPM5_E5 and TRPM5_E22) that may be copy number gains or losses. Therefore, it is unlikely copy number variations in TRPM5 are associated with thermal taster status.

Other potential genes for future studies could be other temperature-sensitive ion channels such as TRPM8, which is cold-sensitive, and TRPM4, which is heat-sensitive (Voets et al., 2004). However, TRPM4 is known to have 40% similarity in the protein sequence to TRPM5 (Voets et al., 2004) and may not be associated with thermal taster status based on the findings here. Another possibility for thermal taster status could be variations in the central nervous system processes, as thermal tasters have also been found to have stronger senses of smell and increased retronasal stimulation by vanillin (Bajec & Pickering, 2008; Green et al., 2005). Therefore, further study is necessary to determine the cause of thermal taste and the mechanism behind thermal taste transduction.

Summary and Perspectives

Of all the associations studied, only those with TAS2R38 were significant for intensity ratings for astringency, sweet taste, bitter taste, sour taste, and PROP taster status. However, the association between TAS2R38 and PROP taster status can explain only 41% of PROP taster status determination, suggesting there are other factors contributing to PROP taster status, such as fungiform papillae density and other potential genes. Although much research into PROP taster status has been conducted, the complete mechanism is still unknown. It is hypothesized that other genes must be

contributing to PROP non-tasting since subjects with the TAS2R38 taster genotypes were classified as PROP non-tasters. Additionally, re-examination of the association between Gustin and PROP taster status is necessary to determine if the association found previously is ethnicity-dependent. Lastly, all future studies should include fungiform papillae density analysis to further explain the contribution to PROP taster status.

The data from this study confirmed that thermal taster status was not associated with PROP taster status or with TRPM5 through the two SNPs and copy number variations studied. This leaves many questions still to be addressed: what is responsible for thermal taster status; is it a genetic or a central nervous system process; and what are the potential health implications of these associations? For future study, it may be necessary to further classify thermal tasters into different subtypes by the temperature cycle and the taste experienced, as different molecular pathways may be involved for different types of thermal taste.

In conclusion, many other factors should be taken into consideration in future taste genetic studies such as ethnicity, learned behaviors related to taste, and fungiform papillae density in order to further the understanding of the mechanism behind PROP and thermal taster status.

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SECTION VI: APPENDICIES

Appendix I: Raw average ratings for sweet, bitter, sour, umami and astringency.

Table A: Raw average ratings for sweet, bitter, sour, umami and astringency.

Subject	Sweet	Bitter	Sour	Umami	Astringency
125	34	46.8	34.8	54.5	41.3
141	53	47.8	38.3	42	45.5
175	13	9.3	4	16.5	13.5
183	49	70	44	51.3	55
191	70	38	43.5	63	43
201	43.8	30.8	48.5	55	41.5
210	13.5	26	17.8	11.8	16.8
221	53.8	72	49.5	78.3	49.5
229	81.3	7.3	33.5	68.3	49.5
260	34.3	5.8	42.3	34	33.3
310	32.5	24.5	23.3	29	44.3
336	19	2.3	5	20	2.5
338	42.8	76.5	77	64	35.5
341	23.5	38.5	24.5	33.5	33
353	75	48.3	77.3	66.3	54.5
379	47	69.3	19	50	16.8
400	29.5	62.3	68.8	29	28.8
413	34.3	33.8	34.5	48.8	42
417	37.8	19.8	44.5	80	15.3
437	64.5	83.3	27.8	70.3	80.3
446	28.8	7.8	21.5	21.5	16.5
447	32	76.3	21.3	32	51.3
464	51	45.8	48.5	86.5	49.5
465	79.8	85.8	55	92.8	26
489	36.5	58.8	50	43.8	50.5
497	50.3	58	41.8	63.5	60.3
499	38.3	33.5	22.3	33.3	33.5
513	48.8	53	61.3	43.5	43
526	22	27.3	17	25	19.8
538	49.5	26.3	4.8	34.5	24.8
559	57	61	24.5	72.5	26.5

Subject	Sweet	Bitter	Sour	Umami	Astringency
600	72.5	38.3	38.8	51.5	67.5
606	65.5	58.8	44.5	70.5	60
680	53.5	43	15.8	25	23.5
723	24	48.5	16.5	33.5	26.5
779	61	55	47.5	21.8	25.5
797	29	44.5	26.3	35.3	46.5
885	38.3	71.5	67.5	19	21.5
894	43.5	33.3	45	70.3	24.5
899	37.5	38.5	62.5	62.8	73.5
948	37	10	29	47	20.5
970	53.3	41	63	91	76.5
988	11	11.8	8	92.3	31
994	31	8.5	17	24	18
1000	81	3	60	69	47.5
1001	27	32	21	41.5	29
1002	58	60	67.5	32	63.5
1003	37.5	62.5	100	62.5	75.5
1004	56	80	50	32.5	44.5
1005	86	90	76.5	82.5	78
1006	47.5	47.5	56.5	52.5	65.5
1007	71	75	39	62.5	66.5
1008	45.5	29	43	27.5	49.5
1009	34	51	51.5	72	56.5
1011	63.5	7	57.5	29	49
1012	21.5	19.5	17	10.5	21
1014	15	49.5	47	58	65.5
1015	63	37	66.5	56.5	59.5
1016	100	100	75	75.5	50
1018	12	14	6.5	17.5	5
1019	31.5	50	49.5	13	75
1020	57.5	48.5	59.5	61.5	65.5
1022	45	35.5	35	58	16
1023	77	80	63	100	54
1024	50.5	65.5	48.5	43	30

Appendix II: Statistical results for analysis of association between SNPs and taste perception (only those not shown in the result section)

Table B1: Result of statistical analysis for the association between SNPs and umami.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs713598	0.17	0.00	0.6804	0.06	0.6804
rs1726866	0.58	0.01	0.4505	-0.10	0.4505
rs10246939	0.26	0.00	0.6137	-0.07	0.6137
rs713598 and rs1726866	1.69	0.06	0.1937		
rs713598 and rs10246939	0.97	0.03	0.3869		
rs1726866 and rs10246939	0.46	0.02	0.6364		
rs713598, rs1726866 & rs10246939	1.14	0.06	0.342		
rs3986599	1.97	0.12	0.1819	-0.35	0.1819
rs34364959	2.28	0.12	0.1495	0.34	0.1495
rs3986599 & rs34364959	2.55	0.28	0.1159		

Table B2: Result of statistical analysis for the association between SNPs and sweet.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs713598	0.42	0.01	0.5185	0.09	0.5185
rs1726866	2.20	0.03	0.1607	-0.19	0.1607
rs10246939	1.74	0.03	0.192	-0.17	0.192
rs1726866 & rs10246939	0.99	0.03	0.3772		
rs3986599	3.81	0.21	0.0712	-0.46	0.0712
rs34364959	0.57	0.03	0.4587	0.18	0.4587
rs3986599 & rs34364959	2.10	0.24	0.1621		

Table B3: Non Result of statistical analysis for the association between SNPs and sour.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs713598	0.06	0.00	0.8093	0.03	0.8093
rs1726866	1.68	0.03	0.2003	-0.17	0.2003
rs10246939	1.92	0.03	0.1716	-0.18	0.1716
rs713598 and rs1726866	3.13	0.10	0.0516		
rs1726866 and rs10246939	0.94	0.03	0.3962		
rs713598, rs1726866 & rs10246939	2.34	0.12	0.0832		
rs3986599	1.06	0.07	0.3205	-0.27	0.3205
rs34364959	1.78	0.09	0.1995	0.31	0.1995
rs3986599 & rs34364959	1.40	0.18	0.2806		

Table B4: Result of statistical analysis for the association between SNPs and bitter.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs713598	0.57	0.01	0.4545	0.10014	0.4545
rs1726866	1.84	0.0318	0.1807	-0.1782	0.1807
rs10246939	1.97	0.0341	0.1655	-0.1845	0.1655
rs1726866 and rs10246939	0.98	0.0343	0.3833		
rs713598, rs1726866 & rs10246939	4.47	0.1988	0.0071		
rs3986599	1.25	0.082	0.2822	-0.2864	0.2822
rs34364959	1.2	0.0661	0.2878	0.25719	0.2878
rs3986599 & rs34364959	1.47	0.1843	0.2661		

Table B5: Result of statistical analysis for the association between SNPs and astringency.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs713598	0.02	0.00	0.8817	-0.02	0.8817
rs1726866	2.72	0.05	0.1049	-0.25	0.1049
rs10246939	3.17	0.05	0.0803	-0.23	0.0803
rs1726866 and rs10246939	1.56	0.05	0.2194		
rs713598, rs1726866 & rs10246939	2.51	0.12	0.0684		
rs3986599	1.02	0.07	0.3272	-0.26	0.3272
rs34364959	1.84	0.10	0.1922	0.31	0.1922
rs3986599 & rs34364959	1.65	0.20	0.2293		

Table B6: Result of statistical analysis for the association between rs2274333 and thermal taster status.

SNP(s)	F-value	R-Squared	p-value	Correlation Coefficient	p-value
rs2274333	0.64	0.0144	0.4376	0.11984	0.4276

Appendix III: Normalized rating for orosensory sensations

Table C: Normalized ratings of all samples for bitter, sweet, umami, sour and mouth-feel astringency*.

Subject	nASTR.	nUMAMI	nSOUR	nSWEET	nBITTER
125	31.6	41.7	26.6	26	35.8
141	31.5	29.1	26.5	36.7	33
175	31.9	39	9.4	30.7	21.9
183	41.8	38.9	33.4	37.2	53.2
191	60.4	88.4	61.1	98.3	53.3
201	45.4	60.2	53.1	47.9	33.7
210	40.1	28.1	42.5	32.3	62.2
221	45.2	71.5	45.2	49.1	65.8
229	26.5	36.5	17.9	43.5	3.9
260	38.3	39.2	48.7	39.5	6.6
310	53.6	35.1	28.2	39.4	29.7
336	6.9	55.3	13.8	52.6	6.2
338	19.2	34.6	41.7	23.1	41.4
341	52.8	53.6	39.2	37.6	61.6
353	48	58.3	68	66	42.5
379	11.3	33.9	12.9	31.8	46.9
400	35.5	35.8	84.8	36.4	76.8
413	32.2	37.3	26.4	26.2	25.8
417	21.6	113.1	62.9	53.4	27.9
437	57.8	50.6	20	46.5	60
446	10.6	13.8	13.8	18.4	5
447	35.5	22.1	14.7	22.1	52.8
464	38.7	67.6	37.9	39.8	35.7
465	31.5	112.3	66.6	96.6	103.8
489	35.7	30.9	35.4	25.8	41.5
497	65.6	69.1	45.4	54.7	63.1
499	39.1	38.8	26	44.6	39.1
513	54.8	55.4	78.1	62.1	67.5
526	18	22.8	15.5	20.1	24.9
538	18.8	26.2	3.6	37.6	19.9
559	38.3	104.8	35.4	82.4	88.2
573	67.8	51.7	38.9	72.8	38.4
600	52.8	62.1	39.2	57.7	51.7
639	25.7	27.4	17.2	58.6	47.1
723	18.6	23.5	11.6	16.8	34
779	20.5	17.5	38.2	49	44.2
797	37.2	28.2	21	23.2	35.6
885	18.3	16.2	57.6	32.6	61
894	25.2	72.4	46.4	44.8	34.3

Subject	nASTR.	nUMAMI	nSOUR	nSWEET	nBITTER
899	55.6	47.5	47.3	28.4	29.1
948	19.9	45.5	28.1	35.8	9.7
970	44.8	53.3	36.9	31.2	24
988	28.2	83.9	7.3	10	10.7
994	16.4	21.9	15.5	28.3	7.8
1000	44.2	64.3	55.9	75.4	2.8
1001	58.5	83.7	42.4	54.5	64.6
1002	58	29.2	61.7	53	54.8
1003	215.1	178.1	284.9	106.8	178.1
1004	359.2	262.3	403.6	452	645.7
1005	83.9	88.8	82.3	92.6	96.9
1006	55.7	44.6	48	40.4	40.4
1007	134.2	126.1	78.7	143.3	151.3
1008	55.8	31	48.4	51.2	32.7
1009	52.6	67.1	48	31.7	47.5
1011	91.3	54	107.1	118.3	13
1012	23.7	11.8	19.1	24.2	22
1014	45.3	40.1	32.5	10.4	34.2
1015	80	76	89.5	84.8	49.8
1016	73.4	110.8	110.1	146.8	146.8
1018	14.2	49.9	18.5	34.2	39.9
1019	181.6	31.5	119.9	76.3	121.1
1020	58.7	55.2	53.4	51.6	43.5
1022	11.2	40.7	24.6	31.6	24.9
1023	174.4	322.9	203.4	248.6	258.3
1024	30.3	43.4	48.9	51	66.1

*Normalization was performed using the average rating for each modality divided by the individual's "brightness of sun rating."

Appendix IV: PROP taster status classifications and ratings

Table D: PROP taster status (PTS), PROP rating, normalized PROP ratings (nPROP) and normalized PTS (nPTS) for all subjects.

Sample	PTS	PROP rating	Average Sun Rating	nPROP	nPTS
125	PMT	43.5	63.3	27.9	PMT
141	PMT	21.5	53	17.1	PMT
175	PST	52	31	70.6	PST
183	PNT	0	46	0	PNT
191	PST	52	12	182.4	PST
201	PNT	18	35	21.7	PMT
210	PNT	18	15.5	48.9	PMT
221	PNT	7	52	5.7	PNT
229	PNT	0	87	0	PNT
260	PST	67	34	83	PST
336	PMT	38	22	72.7	PST
338	PST	100	85	49.5	PMT
341	PNT	14.5	17	35.9	PMT
353	PST	69	55	52.8	PMT
379	PNT	1	47	0.9	PNT
400	PST	52.5	10.5	210.5	PST
413	PST	64.5	47.5	57.2	PMT
437	PST	55.5	65	35.9	PMT
446	PNT	4	71	2.4	PNT
447	PNT	7.8	59	5.5	PNT
464	PST	59.3	45	55.4	PMT
465	PST	95	27	148.1	PST
489	PMT	35	52	28.3	PMT
497	PST	52	47.5	46.1	PMT
499	PMT	42.5	25	71.6	PST
513	PST	71	23	130	PST
526	PST	59.8	46	54.7	PMT
538	PST	55	41.5	55.8	PMT
559	PST	58.3	20	122.6	PST
573	PNT	16.3	76.5	8.9	PNT
600	PNT	12.5	36	14.6	PMT
606	PNT	11	47	9.9	PNT
723	PST	57.5	64	37.8	PMT
779	PST	53	49	45.5	PMT
885	PNT	8.5	54.5	6.6	PNT
894	PNT	0.5	20	1.1	PNT
899	PST	85.3	52	69	PST
948	PMT	29	50	24.4	PMT
970	PST	83.8	73.5	48	PMT

Sample	PTS	PROP rating	Average Sun Rating	nPROP	nPTS
988	PNT	1.5	25.5	2.5	PNT
994	PMT	49.5	53	39.3	PMT
1001	PMT	49	24	86	PST
1002	PST	95	53	75.5	PST
1003	PST	100	17	247.6	PST
1004	PMT	47	6	329.8	PST
1005	PST	100	45	93.6	PST
1006	PST	59	57	43.6	PMT
1007	PST	82	24	143.8	PST
1008	PST	85	43	83.2	PST
1009	PST	58	52	47	PMT
1012	PNT	14	43	13.7	PMT
1014	PNT	8	70	4.8	PNT
1015	PST	82	36	95.9	PST
1016	PNT	16	33	20.4	PMT
1018	PNT	6	17	14.9	PMT
1019	PMT	35	20	73.7	PST
1020	PST	65	54	50.7	PMT
1022	PST	58	69	35.4	PMT
1023	PST	97	15	272.2	PST
1024	PST	91	48	79.8	PST

Appendix V: Absolute peak heights for upQMPSF for TRPM5 and surrounding regions for association with thermal taster status

Table E: Thermal taster status with tastes experienced and the absolute peak heights for upQMPSF for the TRPM5 region.

Subject	TTS & Taste*	3q	11q	Control1	TSPAN32	TRPM5_E3	TRPM5_E22	Marker1	KCNQ1
141	TT b/sa	839	1713	1424	561	472	32.5	1336	154
183	TT b	529	763	490	395	270	69	527	211
201	TT sw/b/sa	281	399	262	203	121	42.5	270	144
210	TT sw/b/sa	842	1124	858	504	393	126	882	388
221	TT sw/sa/so	189	270	236	134	111	46	185	85
229	TT sa/so	549	1253	1108	622	496	120	1073	451
336	TnT	2532	4091	3060	2402	1136	253	2551	1067
338	TT b	861	1374	924	726	522	133	1110	521
341	TnT	338	524	329	216	99	32	284	194
379	TT b	273	412	272	199	115	49	280	143
400	TnT	573	882	621	503	389	103	746	320
413	TT sw	660	846	663	424	268	75	736	244
420	TnT	398	504	322	195	120	37.5	292	124
437	TT b/sa/so	608	721	508	283	105	36	363	169
446	TnT	543	882	563	574	323	72	635	196
447	TT sw	1329	2361	1437	1518	1107	218	2067	930
464	TnT	798	1355	924	902	694	160	1197	404
465	TnT	597	1490	1223	1038	709	157	1214	365
489	TnT	888	1516	1027	1104	934	242	1317	468
499	TT so	724	1176	877	729	585	149	1070	427
513	TT sw/so	276	409	285	239	156	44	343	172
526	TT b/sa	500	717	516	432	270	87	656	238
538	TnT	870	1338	975	739	441	105	1041	412
559	TnT	705	972	744	608	461	137	917	335

Subject	TTS & Taste*	3q	11q	Control1	TSPAN32	TRPM5_E3	TRPM5_E22	Marker1	KCNQ1
573	TnT	1572	2030	1652	1038	804	224	1767	638
600	TT b	1020	1506	1179	847	551	117	1263	419
639	TT NA	211	350	235	148	133	0	215	69
723	TnT	1325	1846	1344	1209	819	196	1479	463
779	TnT	1137	1792	1085	694	424	123	1204	513
885	TT b	768	1301	877	735	482	97	1063	543
894	TT so	262	380	253	187	69	32	235	125
899	TT so	1416	2131	1565	1327	698	148	1603	516
948	TnT	3510	5080	4007	2774	2092	538	4649	1202
970	TT b/so	926	1205	891	564	262	85	871	410
994	TnT	1082	1186	1219	720	516	132	1133	357
1003	TT so	2727	3845	3067	1908	1047	313	2104	394
1004	TT b	3039	5375	4087	2796	1925	621	3365	767
1005	TnT	1609	2526	2014	1560	863	240	1782	452
1006	TT so	2548	3631	2936	1778	872	221	2098	504
1008	TnT	1803	3094	2323	1905	1139	323	2049	472
1012	TT b/so	1253	2151	1445	1304	1025	324	1650	368
1014	TnT	2027	3303	2412	1833	1062	310	1716	481
1016	TnT	1906	3547	2285	2181	1603	465	2614	706
1020	TT sw	1367	2791	1640	1988	1658	294	2377	787
1022	TT so	2449	3916	2404	2235	1339	283	2546	645

*Tastes experienced on heating or cooling trials; bitter (b), sweet (sw), sour (so), salty (sa).

Appendix VI: Multiple Sequence Alignments for protein sequence regions containing the selected SNPs.

rs2233998

```
TAS2r4_clf      MLQIFFLSAIIFSAILNFVGLIVNLFIAVVSYRTWLKSHRISSSNWILFSLGITRCLMLG       60
TAS2R4_bt       MLRIVFFSSVVVSEILTFVGLIVNLFIVVVSYKTCIKSHRISSSDRLLFSLGITRFFIL-
TAS2R4_hs       MLRLFYFSAIIASVILNFVGIIMNLFITVVNCKTWVKSHRISSSDRILFSLGITRFLMLG
TAS2r4_pt       MLRLFYFSAIIASVILNFVGIIMNLFITVVNCKTWVKSHRISSSDRILFSLGITRFLMLG
TAS2r4_gg       MLRLFYFSAVIASVILNFVGIIMNLFITVVNCKTWVKSHRISSSDRILFSLGITRFLMLG
TAS2r4_mm       MLWLFHFSAIIASVILNFVGIIMSLFITVVNYKTWVKSHRISSSERILFSLGITRFFMLG
TAS2R4_mmu      MLWELYVFVFAASVFLNFVGIIANLFIIVIIIKTWVNSRRIASPDRILFSLAITRFLTLG
                **  ..:.  .  * :*.***:* .*** *:  :* ::*:**:*.: :****.*** : *
```

rs2234001

```
TAS2r4_clf      LFLLNIIYFFISPKMERSVHLSHFFLSXWMFLDSNSLWFVTLLIALYCVKITDFQLGVFL       120
TAS2R4_bt       ---LLNVVVIISPNMERSVSLSYFFLSCWMFLDCSSLWFVTLLNVLYCVKIANYQHSVFL
TAS2R4_hs       -LFLVNTIYFVSSNTERSVYLSAFFVLCFMFLDSSSVWFVTLLNILYCVKITNFQHSVFL
TAS2r4_pt       -LFLVNTIYFVSSNXERSVYLSAFFVLCFMFLDSSSLWFVTLLNILYCVKITNFQHSVFL
TAS2r4_gg       -LFLVNTIYFVSSNMERSVYLSAFFVLCFMFLDSSSLWFVTLLNILYCVKITNFQHSVFL
TAS2r4_mm       -LFLVNTIYFVSSNKERSVYLSAFFVLCFMFLDSSSLWFVTLLNSLYCVKITNFQHSVFL
TAS2R4_mmu      --LFLLNSVYIATNTGRSVYFSTFFLLCWKFLDANSLWLVTILNSLYCVKITNFQHPVFL
                   :      ::.:  *** :* **:  : ***..*:*:**:*  ******:::*  ***
```

Figure F1: **Multiple protein sequence alignment for TAS2R4 protein region corresponding to rs2233998 and rs2234001**. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_058640.1, *Pan troglodytes* (pt) NP_001009161.1, *Macaca mulatta*(mm) XP_001084778.1, *Gorilla gorilla* (gg) Q645Y8.1, *Canis lupus familiarilis* (clf) XP_003639608.1, *Bos Taurus* (bt) NP_001040100.1, and *Mus musculus* (mmu) NP_065248.1. The amino acid residue impacted by the SNP is labeled in yellow highlight, and this format applies to all alignment figures.

```
TAS2R13_hs      YASFFLCVLISWIS-ELYQNTVIYMLCETIGVFSPSSHSFLLILGNAKLRQAFLLVAAKV      300
TAS2R13_gg      YASFFLCVLXSWIS-ELYQNTVIYMLCETIGVFYPSSHSFLLILGNAKLRQAFLLVAAKV
TAS2R13_pt      YASFFLSILISWIS-ELYQNTVIYMLCETIGAFYPSSHSFLLILGNAKLRQAFLLVAAKV
TAS2R13_mm      YASFFLCILISWIS-ELYQNILIHMFCQTIGVFYPSSHSFLLILGNPKLRQASLLVAAKV
TAS2R13_mmu     YATYFISFFLSLIP-MAHKTRLGLMFSITVGLFYPSSHSFILILGHSNLRQASLWVMTYL
TAS2R43_clf     LVCYFLTLIAIVWSSNRLQNKLIFLLCKAIGILYPSSHSFILIWGNKKLREDFLSFLWQL
TAS2R46_bt      FATYMLTVILTIWNSNELQKELVQMLFQALAITYPSIHSFILIWTNRKLTQTFLSFLWQP
                 . ::: .:         :. :  ::  ::.   ** ***:**  : :* :  * .
```

Figure F2: A **Multiple protein sequence alignment for TAS2R13 protein region corresponding to rs1015443**. The species and sequence accession numbers of the proteins used include, *Homo sapiens* (hs) NP_076409.1, *Pan troglodytes* (pt) NP_001009141.1, *Macaca mulatta*(mm) NP_001074236.1, *Gorilla gorilla* (gg) Q645Z5.1, *Canis lupus familiarilis* (clf) NP_001138979.1, *Bos Taurus* (bt) XP_001251311.1, and *Mus musculus* (mmu) NP_996907.1.

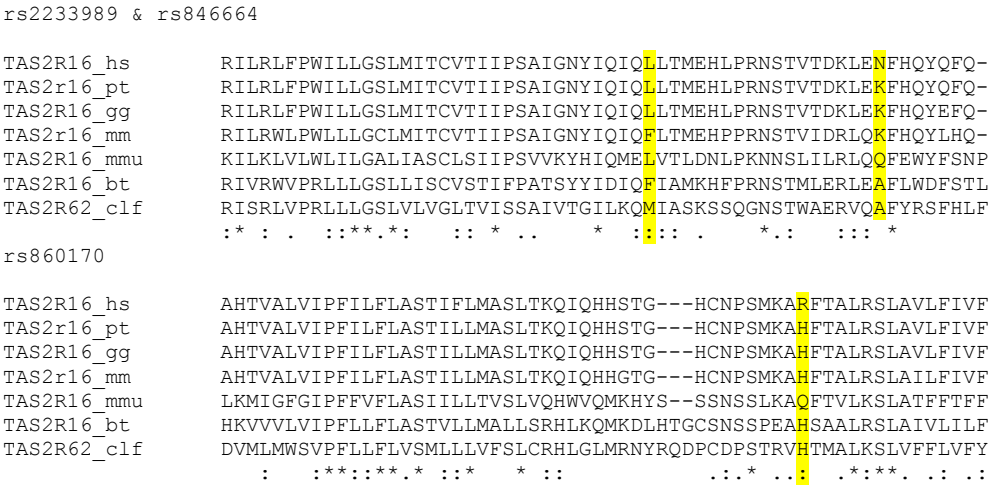


Figure F3: Multiple protein sequence alignment for TAS2R16 protein region corresponding to rs2233989, rs846664 and rs860170. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_058641.1, *Pan troglodytes* (pt) NP_001009160.1, *Macaca mulatta*(mm) NP_001074228.1, *Gorilla gorilla* (gg) Q645Y4.1, *Canis lupus familiarilis* (clf) XP_003432137.2, *Bos Taurus* (bt) NP_001040093.1, and *Mus musculus* (mmu) NP_996905.1

```
rs12578654

TAS2R43_clf       VILINWYATLLNPALYSLEVRLLVHIAWTANNHFSIWLATSLSVFYLFKIANFSNLIFLR     120
TAS2R46_bt        TILINWYATMYNPALY--SLRIVIRVAWTVSNHFSNWLATSLSIFYLFKIANFSSLIFLH
TAS2r19_hs        VMLFLWYATVFNSALYGLEVRIVASNAWAVTNHFSMWLAASLSIFCLLKIANFSNLISLH
TAS2R48_gg        VMLFLWYATVFNSALYGLEVRIVASNAWAVMNHFSMWLAAILSIFCLLKIANFSNLIFLH
TAS2R19_pt        VMLFLWYATVFNSALYGLEVRIVASNAWAVMNHFSIWLAASLSIFCLLKIANFSNLIFLH
TAS2R64_mm        AILLYWYATMFNSALCSSEVRIFASNISAIINHFSIWLAASLSIFYLLKIANFSNLIFLH
TAS2R120_mmu      AMLTNWHSHVFTPDTDNLQMRVFGGITWAITNHFTTWLGTILSMFYLFKIANFSNSLFLH
                  .:*  *:: : ..     .:*:.     :  ***: **.: **:* *:******. : *:

rs1868769

TAS2R43_clf       LKWRVKSVVFVMLLGSLFFLVFHVAVVSIYEQMQMKEYEGNITRQTKLRDIAQLMNMTVF     180
TAS2R46_bt        LKWRVKSVVLMMILGTSVILFFQVAVLSIDETIQTSEYERNITEKTKLRDILHLSNMTLL
TAS2r19_hs        LKKRIKSVVLVILLGPLVFLICNLAVITMDERVWTKEYEGNVTWKIKLRNAIHLSSLTVT
TAS2R48_gg        LKKRIKSVVLVILLGPLVFLICNLAVITMDERVWTKEYEGNVTWKIKLRNAIQLSNLTVT
TAS2R19_pt        LKKRIKSVVLVILLGPLVFLICNLAVITMDERVWTKEYEGNVTWKIKLRNAIQLSNLTVT
TAS2R64_mm        LQKRIKSVVRVMLLGPLVFLICNLAVVTTDEGVWTKEYEGNVTWKIKLKNAIHLSNLTIS
TAS2R120_mmu      LKRKLDNVLLVIFLGSSLFLVAYLGMVNIKKIAWMSIHEGNVTTKSKLKHVTSITNMLLF
                  *: ::..*: :::**. .:*.  :.:::.  :    . :* *:* : **:.   : .: :
rs10772420

TAS2R43_clf       YFLTLIAIVWSSNRLQNKLIFLLCKAIGILYPSSHSFILIWGNKKLREDFLSFLWQLKGW    300
TAS2R46_bt        YMLTVILTIWNSNELQKELVQMLFQALAITYPSIHSFILIWTNRKLTQTFLSFLWQPRCW
TAS2r19_hs        YFLCIITSTWNLRTQQSKLVLLLCQTVAIMYPSFHSFILIMGSRKLKQTFLSVLWQMTR-
TAS2R48_gg        YFLCIITSTWNLRTQQSKLVLLLCQTVAIMYPSFHSFILIMGSRKLKQTF----------
TAS2R19_pt        YFLCIITSTWNLRTQQSKLVLLLCQTVAIMYPSFHSFILIMGSRKLKQTFLSVLWQMTC-
TAS2R64_mm        YSLCLISLTWSPWKQQNKLVFLLCQTLAIMYPSFHSFILIRGNRKLK-----------
TAS2R120_mmu      YSSCVIISGWS---LQNAPVFLFCVTIGSFYPAGHSCILIWGNQKLKQVFLLLLRQMRC-
                  *   :*   *.    *.  : ::  ::.  **: ** ***  .:**
```

Figure F4: Multiple protein sequence alignment for TAS2R19 protein region corresponding to rs12578654, rs1868769, and rs10772420. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_795369.1, *Pan troglodytes* (pt) XP_003313544.1, *Macaca mulatta*(mm) BAD98135.1, *Gorilla gorilla* (gg) BAD98092.1, *Canis lupus familiarilis* (clf) NP_001138979.1, *Bos Taurus* (bt) XP_001251311.1, and *Mus musculus* (mmu) NP_996906.1.

```
TAS2R43_clf       TLMNFVPFAISLTSFLLLIFSLWKHLKKMRSGGKRYQDSSTKVHIKAMQTVISFLLLLVC     240
TAS2R46_bt        TLTNFIPFTMSLVSFLLLIFFLWKHLRKMQLNGKRSQDPSTKVHIKAMQTVISFLFLFAT
TAS2R31_hs        TLGNLVPFTLTLLCFLLLICSLCKHLKKMQLHGKGSQDPSTKVHIKALQTVIFFLLLCAV
TAS2R31_pt        TLGNLVPFTLTLLCFLLLICSLCKHLKKMQLHGKGSQDPSTKVHIKVLQTVISFLLLCAI
TAS2R31_gg        TLGNLVPFTLTLLCFLLLICSLCKHLKKMQLHGKGSQDPSMKVHIKALQTVTSFLLLCAI
TAS2R43_mm        MLANLVPFTLTLLSFVLLIWSLCKHLKKMQLYGKGSQDPSTKVHIKALQTVISFLFLCAI
TAS2R120_mmu      SLINIVPFGISLNCVLLLIYSLSKHLKNMKFYGKGCQDQSTMVHIKALQTVVSFLLLYAT
                   * *::** ::* ..:***  * ***::*:  **  ** *  ****.:***  **:* .
```

Figure F5: Multiple protein sequence alignment for TAS2R31 protein region corresponding to rs10845293. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_795366.2, *Pan troglodytes* (pt) XP_001151214.2, *Macaca mulatta*(mm) XP_001082472.1, *Gorilla gorilla* (gg) Q645Z6.1, *Canis lupus familiarilis* (clf) NP_001138979.1, *Bos Taurus* (bt) XP_001251311.1, and *Mus musculus* (mmu) NP_996906.1

```
rs713598

TAS2R38_bt       MVTLTHIVSVPSEVRNAFLFFSVLEFAVGILLNAFIFLVNFRDLVRRQPLSHCDLVLLSL   60
TAS2R38_clf      ------------------MFLSVLELAVGILTNAFIFLVNFWDVVRRQPLSNCDLILLSL
TAS2R38_pt       MLTLTRIHTVSYEVRSTFLFISVLEFAVGFLTNAFVFLVNFWDVVKRQPLSNSDCVLLCL
TAS2R38_gg       MLTLTRIRTVSYEVRSTFLFISVLEFAVGFLTNAFVFLVNFWDVVKRQPLSNSDCVLLCL
TAS2R38_hs       MLTLTRIRTVSYEVRSTFLFISVLEFAVGFLTNAFVFLVNFWDVVKRQALSNSDCVLLCL
TAS2R38_mm       MLTLTHICTVSYEVRSTFLFISVLEFAVGFLTNAFISLVNFWDVVKRQPLSNSDCVLLCL
TAS2R38_mmu      MLSLTPVLTVSYEAKISFLFLSAMEFAVGILANAFIVLVNVWDVVKKQPLNNCDIALLCL
                                 :*:*.:*:***:* ***: ***. *:*::*.*.::*  **.*
rs1726866 & rs10246939

TAS2R38_bt       AHTRALRSLVSFFCLYVLSLCAALFSIPLLMLWHSKVGVMVCIGIMAACPSGHAVILISG   300
TAS2R38_clf      AHIKALISLISFLCLYVVSFCVALISVPLTMVWHNKIGVMICVGILAACPSIHAAILISG
TAS2R38_pt       AHIKALKSLVSFFCFFVISSCAAFISVPLLILWRDKIGVMVCVGIMAACPSGHAAVLISG
TAS2R38_gg       AHIKALKSLVSFFCFFVISSCAAFISVPLLILWRDKIGVMVCVGIMAACPSGHAAVLISG
TAS2R38_hs       AHIKALKSLVSFFCFFVISSCVAFISVPLLILWRDKIGVMVCVGIMAACPSGHAAILISG
TAS2R38_mm       AHIKALKSLISFFCFFVISSCAAFISVPLLILWHDKIGVMVCVGIMAACPSGHAAVLISG
TAS2R38_mmu      AHIRAIIFLISFFCFYVVSFCAALISIPLLMLWHNKGGVMICIGMMAACPSGHAAILISG
                 ** :*:  *:**:*::*:* *.*::*:** ::*:.* ***:*:*::***** **.:****
```

Figure F6: Multiple protein sequence alignment for TAS2R38 protein region corresponding to rs713598, rs1726866 and rs10246939. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_789787.4, *Pan troglodytes* (pt) NP_001009139.1, *Macaca mulatta*(mm) AAU21198.1, *Gorilla gorilla* (gg) Q697L4.1, *Canis lupus familiarilis* (clf) NP_001138972.1, *Bos Taurus* (bt) XP_001255055.1, and *Mus musculus* (mmu) NP_001001451.1

```
CA6_hs                VRYNPSLKGLNMTGYETQAGEFPMVNNGHTVQISLPSTMRMTVADGTVYIAQQMHFHWGG
CA6-like_pt           VRYNPSLKGLNLTGYETQAGEFPMVNNGHTVQISLPSTMRMTAADGTVYIAQQMHFHWGG
CA6-like_mm           VRYNPALKGLKLTGYETQEGEFPMVNNGHTVQISLPSTMRMTAADGTVYIAQQMHFHWGG
CA6-precursor_clf     VQYNPSLKALKLTGYRIQVGEFPMINNGHTVQISLPPTMRMMASDGTEYIAQQMHFHWGG
CA6-precursor_bt      VRYNPSLRALNLTGYGLRQGEFPMTNNGHTVQISLPSSMRMTTSDGSQYLAKQMHFHWGG
Unnamed_mmu           VMFNPSLKPLSLVNYEKENLEFTMTNNGHTVSIDLPPSMYLETSDGTEFISKAFHFHWGG
CA6-like_dr           VRYSPRMQQLELTGYEDIRGSFLMKNNGHSVEIQLPSTMKITKGFPHQYTAVQMHLHWGG
                      * :.* :: *.:..*     .* * ****:*.*.**.:* :  .    : :  :*:****
```

Figure F7: Multiple protein sequence alignment for Gustin protein region corresponding to rs2274333. The species and sequence accession numbers of the proteins used include *Homo sapiens* (hs) NP_001206.2, *Pan troglodytes* (pt) XP_003307839.1, *Macaca mulatta*(mm) XP_001099188.2, *Canis lupus familiarilis* (clf) NP_001002999.1, *Bos Taurus* (bt) DAA21262.1, *Mus musculus* (mmu) BAE36243.1, and *Danio rerio* (dr) XP_002666525.1.

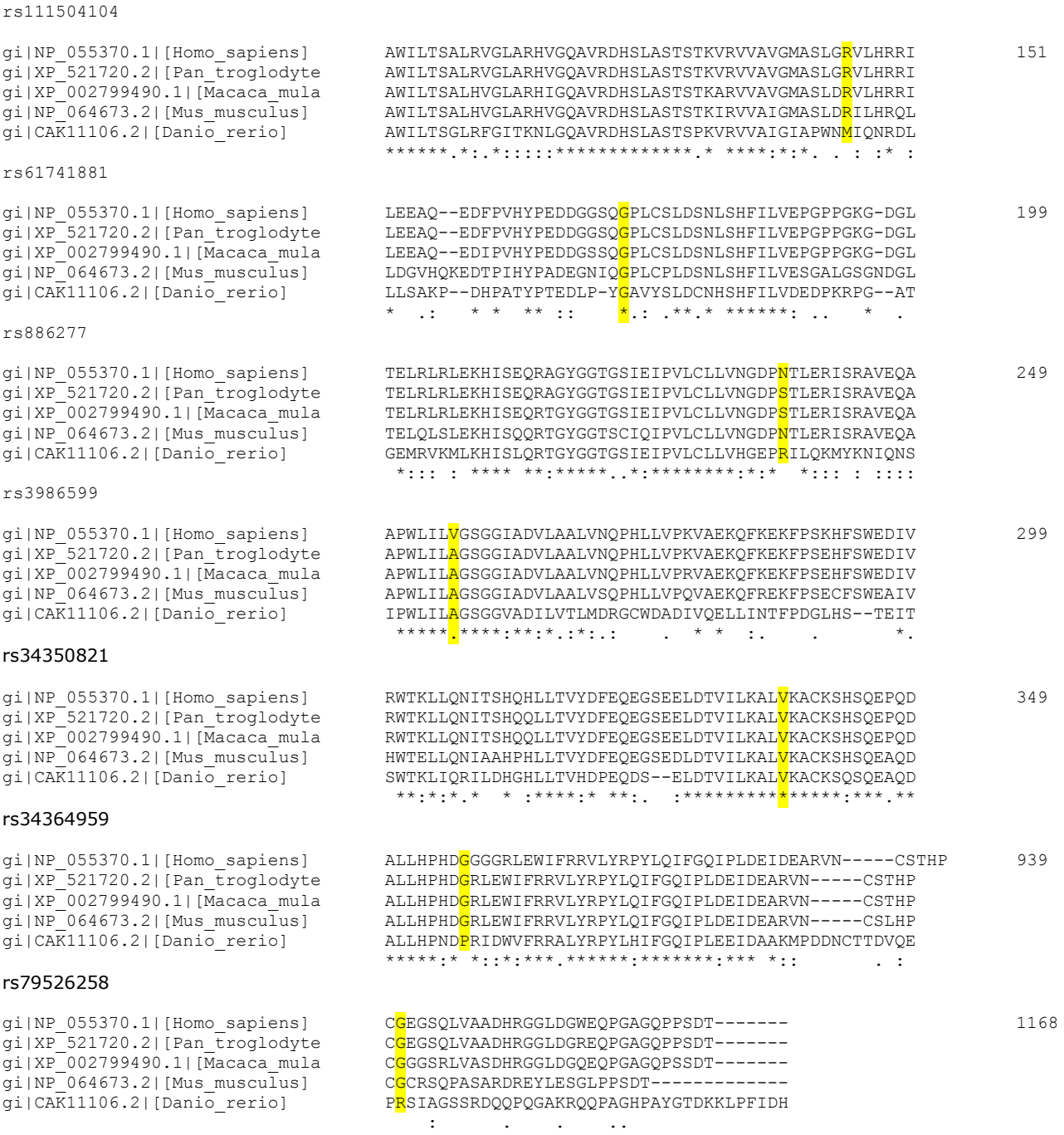


Figure F8: Multiple protein sequence alignment for TRPM5 protein region corresponding to rs79526258, rs34364959, rs34350821, rs3986599, rs886277, rs61741881, and rs111504104. The species and sequence accession numbers of the proteins used include NP_055370.1 *Homo sapiens*, XP_521720.2 *Pan troglodytes*, XP_002799490.1 *Macaca mulatta*, NP_064673.2 *Mus musculus*, CAK11106.2 *Danio rerio*. Amino acid locations are listed to the right of the sequences.

Appendix VII: Consent Form for Sensory Evaluation

INFORMATION STATEMENT AND CONSENT FORM

Name of Research Project: Genetic analysis of variation in human taste perception.

Department/Institute: Brock University; Department of Biological Science/ Cool Climate Oenology and Viticulture Institute

Principal Student Investigator: Ms. Amanda Bering, graduate student, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, ah06ae@badger.ac.brocku.ca

Principle Investigator/Faculty Supervisor: Dr. Ping Liang, Associate Professor, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 5922, pliang@brocku.ca

Co-Investigator: Dr. Gary Pickering, Associate Professor, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4715, gpickeri@brocku.ca

The Project:
The ability to taste the (potentially bitter and mildly unpleasant) compound 6-n-propylthiouracil (PROP), and the perceived intensity of this compound if detected, are largely genetically determined and may vary greatly from individual to individual. Sensitivity to PROP has been related to food preference and acceptance. Likewise, the ability to experience 'taste' sensations as a result of thermal stimulation has also been suggested to be genetically variable, and may have implications in food preference and acceptance. This project examines genetic variations associated with these taste perception variations.

The Procedure:
You are invited to participate in this study! A group of 60 participants that meet defined classification criteria will complete a general demographic, use of alcohol and tobacco, and a health questionnaire. These individuals will also have their PROP taster-status and thermal-taster status determined. PROP-taster status is determined by rating the intensity of a solution of PROP after an orally rinsing with it (and expectorating). Quickly cooling and heating a small area of the tongue tip determines thermal-taster status. These participants will also use a method of measuring perceived intensity over time of sweet, sour, bitter, savory, and astringent stimuli at room temperature. Additionally, a sample of DNA will be taken in a non-invasive procedure (you will rinse with a commercial mouthwash and spit into a sample tube) and analyzed to examine the genes involved in thermal taste.

Benefits/Risks

The expected benefits for the participant and the scientific community are a greater understanding of the role of genetically-mediated indices in taste perception, which in turn may help better understand human habitual dietary intake and susceptibility to chronic diet-related diseases. The determination of thermal taster status is very novel, as such, participants will be among a very few others (less than 300 people world-wide) who have had their thermal-taste status determined, and the first to have their DNA analyzed for this source of individual variation. In addition, participants will gain awareness of their palates, and potentially food preferences.

The expected risks are no great than those encountered in normal daily food and beverage consumption. While they may not be pleasant to everyone, all substances to be tasted are perfectly safe, and are of food-quality grade or better.

Those participants that meet the defined classification criteria will receive one study credit.

Voluntary Participation:

You are free to withdraw your participation in the research at any time, and if you do, any data collected from will immediately be destroyed. For Brock students, your decision whether or to participate in this research will in no way affect your grades or relationship with the investigators.

Responsibilities:

The participant needs only to schedule a 1-hour block of time to come to the CCOVI Sensory Lab (IH301) at Brock University. Times will be available during normal working hours, after working hours, and on weekends, as agreed upon by the participant and the Principle Student Investigator.

Publication of Results

It is expected that the results of this study will be published in academic journals and presented at conferences. Please feel free to contact either the Principle Student Investigator, or the Principle Investigator/Faculty Supervisor at any time should any questions arise. Also, for more information on the progress or results of the study please contact either persons mentioned above, or consult the Liang lab website at: http://edit.brocku.ca/mathematics-science/departments-and-centres/biology/people/ping-liang.

Confidentiality

All data will be confidential, individual identities will not be disclosed to anyone outside of the researchers listed above. Samples (i.e., DNA) collected will be destroyed within 4 months of project completion as de-identified samples bio-hazard garbage according to Brock's related bio-safety policies. Paper data collected in this study (i.e., questionnaires) during this study will be will be

retained for 7 years. Digitally recorded measures (i.e., intensity measures collected via computer) will be retained on disc for 7 years. Data will be stored in a locked, private area accessible only to the Principle Investigator/Faculty Supervisor. Only the individuals listed above will have access to the data.

Ethics Clearance:

This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University (file#: REB - 09-258). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Consent:

The purpose of the research has been explained to me, including the potential risks/discomforts associated with the research. I have also been given the opportunity to ask questions about the research and received satisfactory answers, and know that I may continue to ask questions and receive satisfactory answers throughout the study.

I understand that I am free to withdraw my participation in the research at any time and that if I do I will not be subjected to any penalty or discriminatory treatment; however, I do understand that I will not receive 1 study credit. I also understand my participation in this project is on a voluntary basis, and no remuneration will be provided by Brock University in exchange for my participation.

I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

This study has been reviewed and approved by the Brock University Research Ethics Board (Ethics file#: REB - 09-258). I understand that if I have any complaints or concerns about this research I can contact:
Research Ethics Officer, Office of Research Services, Brock University, Ph: 905 688 5550, ext: 3035; **reb@brocku.ca**

Your Name: _____

Signature: _____

Date: _____

Please check the box below if you **DO NOT** wish to give a DNA sample.

☐

Please check the box below if you **ARE NOT** interested in being contacted to participate in the future studies performed by the Pickering lab. Otherwise, please provide your contact information, which will be solely used for contacting to participate future studies.

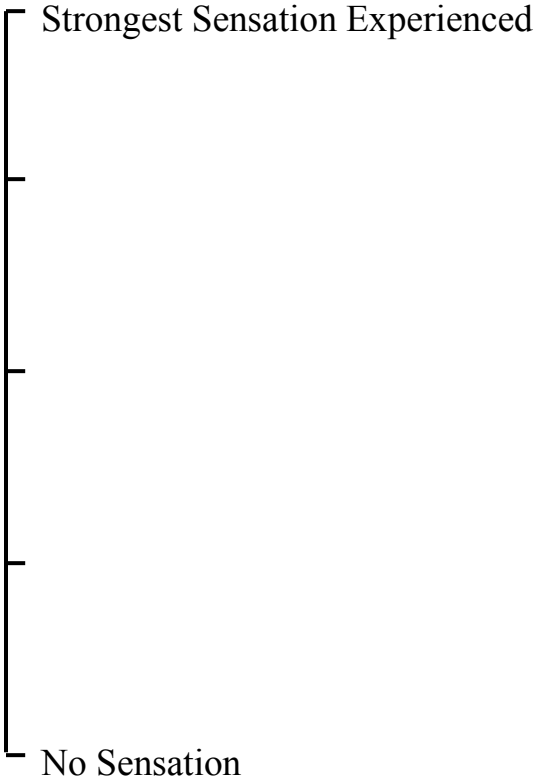
☐ or Email:_____Telephone:

Appendix VIII: gVAS scale for Scale Acclimation for 15 remembered sensations

You are being asked to rate the intensity of a remembered sensation, namely **brightness of the sun when you are looking directly at it**, by indicating where it lies on a scale of all experienced sensations. Please take a moment to think about the last time you experienced this sensation, and how intense it was for you.

The top of the scale is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale is *No Sensation*.

brightness of the sun when you are looking directly at it



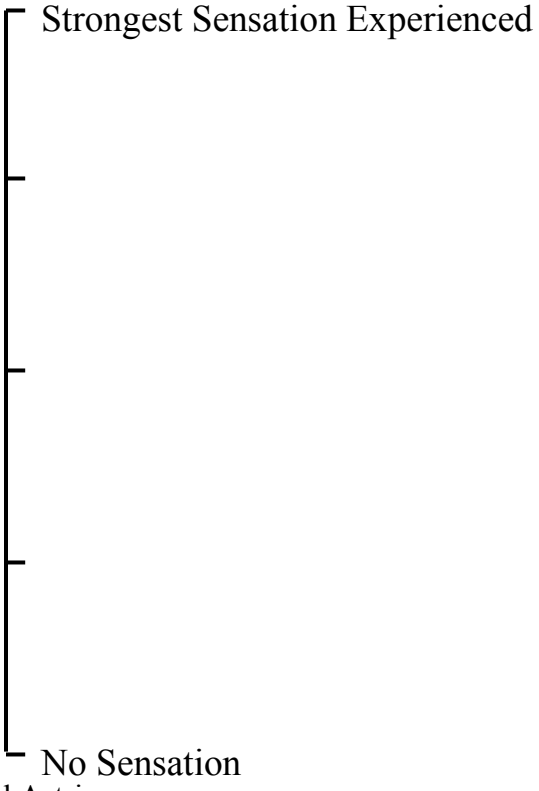
This scale was used for all 15 remembered sensations listed in the Materials and Methods.

Appendix IX: gVAS scale for Basic Taste and Astringency Ratings

Please rinse with the water provided, followed by pectin and again with water. You are being asked to rate the intensity of **bitter** by indicating where it lies on a scale of all experienced sensations. The top of the scale is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale is *No Sensation*.

Please take the entire volume of the sample provided, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit out). After you have expectorated, wait approximately ten (10) seconds then rate the maximum intensity that you perceived in the preceding fifteen (15) seconds. Please keep in mind, that you are rating the **maximum intensity** for bitterness, whenever it may occur. *Please mark the scale with a horizontal line only*.

Bitter



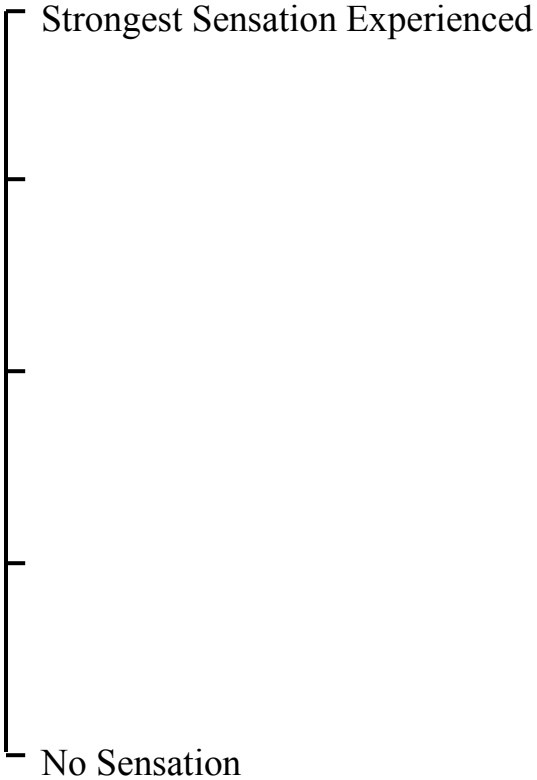
Also used for Sweet, Sour, Umami and Astringency

Appendix X: gVAS scale used for Triple-digit coded Tastants

Please rinse with the water provided, followed by pectin and again with water. You are being asked to rate the intensity of the sample by indicating where it lies on a scale of all experienced sensations. The top of the scale is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale is *No Sensation*.

Please take the entire volume of the sample provided, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit out). After you have expectorated, wait approximately ten (10) seconds then rate the maximum intensity that you perceived in the preceding fifteen (15) seconds. Please keep in mind, that you are rating the **maximum intensity**, whenever it may occur. *Please mark the scale with a horizontal line only*.

Sample ID: **Taste:**



Used for all coded solutions

Appendix XI: gLMS scale used for PROP bitterness Ratings

ID: _____

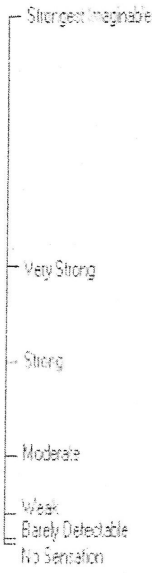
PROP

You are being asked to rate the intensity of a remembered sensation, namely, the **bitterness**, by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*

bitterness



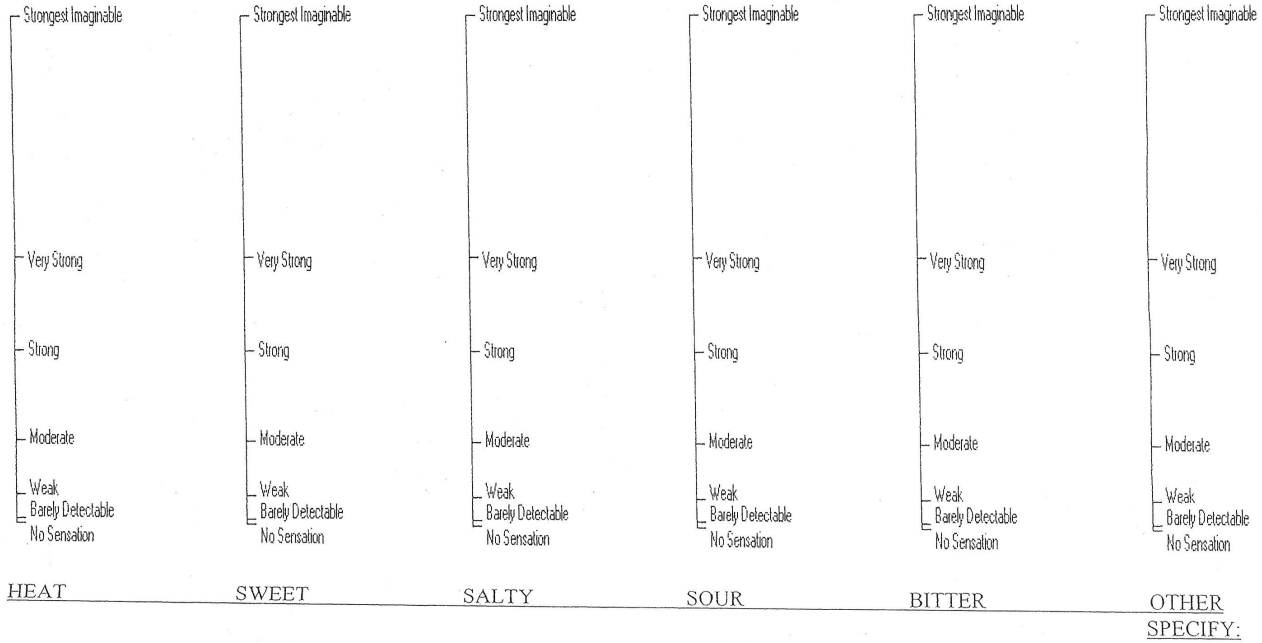
Appendix XII: gLMS scales for Thermal taster status

ID: _____
Location: _____
Rep: _____

You are being asked to rate the intensity the sensations you experienced upon **heating of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is *the strongest sensation of any kind that you can imagine experiencing*.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.

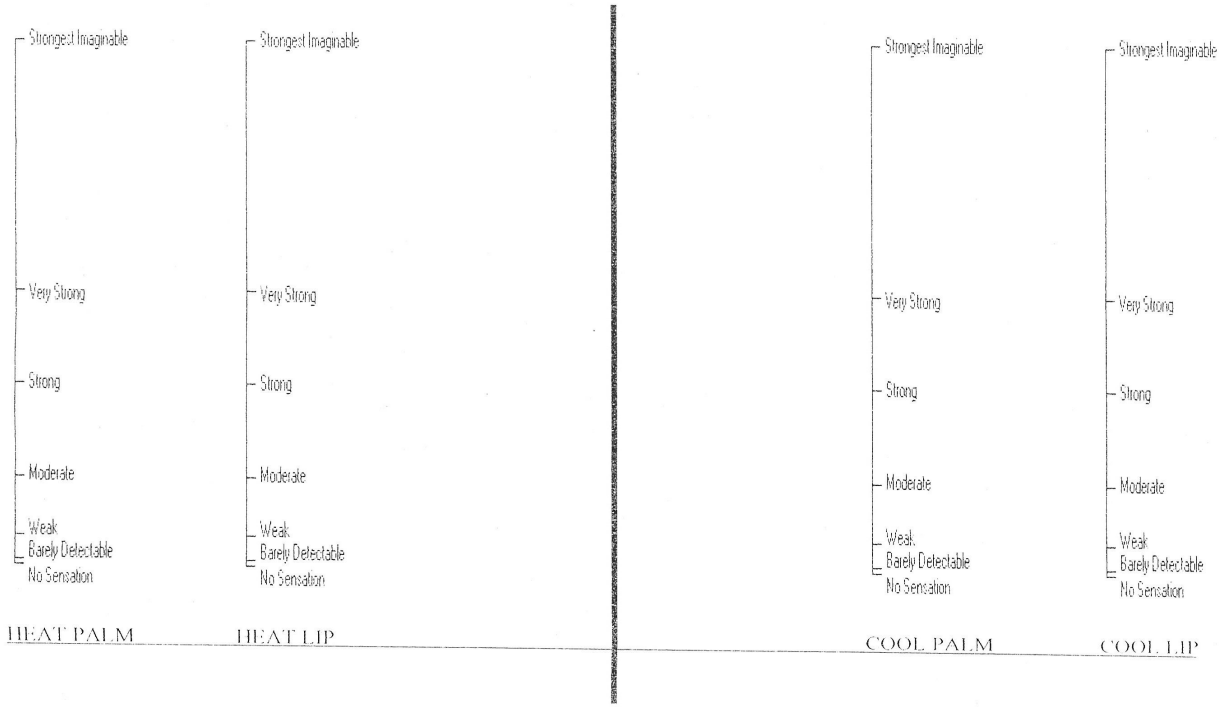


ID: _____
Rep: _____

You are being asked to rate the intensity of the **temperature applied to your lip, and the temperature applied to the palm of your hand** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*

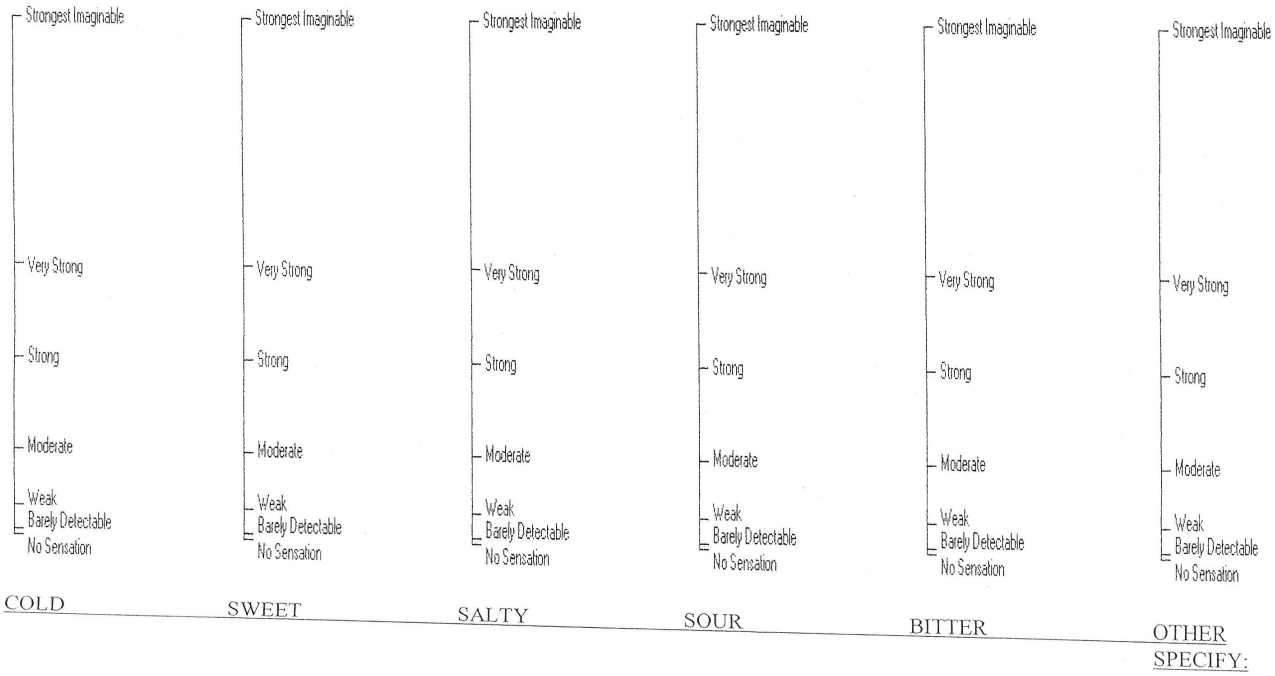


ID: _____
Location: _____
Rep: _____

You are being asked to rate the intensity the sensations you experienced upon **cooling of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is *the strongest sensation of any kind that you can imagine* experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation <u>that you can ever imagine experiencing</u>.



Appendix XIII: Demographics and Health Questionnaire

Demographics

Age:

_____

Gender:

F ☐ M ☐

Ethnicity (please check the one that best applies, if none of these apply, please indicate your ethnicity under 'Other'. If you would like to add any additional information, please do so under the 'Comments' section):

White	☐
Chinese	☐
South Asian	☐
Southeast Asian	☐
Black	☐
Filipino	☐
Japanese	☐
Latin American	☐
Arab	☐
Aboriginal (i.e., North American Indian/Metis/Inuit)	☐
Other (please specify):	☐

_____

Were you born in North America:

Yes ☐ No ☐

If no, where were you born:

City: _____

Country: _____

Do you consume alcoholic beverages?

Yes ☐ No ☐

On average, how many times a month do you drink the following beverages?
(please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
white wine																							
red wine																							
beer																							
spirits																							
other																							
(e.g. cooler)																							

Please indicate, on average, how many of the following beverages you consumer per day.
(**1 drink** = 12 oz. Beer **OR** 6 oz. Wine **OR** 1.5 oz. Spirit)

	#
white wine	
red wine	
beer	

spirits	
other	
(e.g. cooler)	

Do you smoke, or use tobacco products?

Yes ☐ No ☐

If yes, please indicate how many times a month you use the following tobacco products.
(please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
cigarettes																							
cigars																							
pipe																							
snuff																							
chewing tobacco																							

Please indicate, on average, how many of the following tobacco products you use per day
(on days when using tobacco products).

	#
cigarettes	
cigars	
pipe	
snuff	
chewing tobacco	

ID:_____

Health

Please answer the following questions to the best of your ability.

	Yes	No
Do you currently have any diseases, disorders, or illnesses?		
If yes, please list all conditions:		

	Yes	No
Are you currently taking any prescription medications?		
If yes, please list (if you are uncertain of the drug name, please indicate what you are taking it for and its mode of delivery (e.g., inhaler for asthma, pills for gout, cream for eczema, etc.):		

	Yes	No
Are you currently taking any non-prescription medications/drugs regularly (e.g. aspirin, Tylenol, homeopathics, etc.)?		
If yes, please list:		

	Yes	No
Do you have any facial/head piercings?		
If yes, please indicate their location(s) (e.g., ears, tongue, nose, eyebrow, etc.):		

	Yes	No
Have you ever had any oral and/or nasal damage?		
If yes, please indicate their location(s) (e.g., biting through tongue, cheek damage, broken nose, nose surgery, oral surgery, invasive dental procedures (wisdom teeth removed, root canal, etc.):		

	Yes	No
Have you ever had a head injury leading to concussion and/or loss of consciousness?		
If yes, please indicate the number of times you have had a head injury resulting in concussion and/or loss of consciousness:		

Appendix XIV: Emotional Reactivity Survey

DIRECTIONS: The following questions refer to emotional reactions to typical life-events. Please indicate how YOU react to these events by placing a number from the following scale in the blank space preceding each item. Please base your answers on how YOU react, not on how you think others react or how you think a person should react.

Never	Almost Never	Occasionally	Usually	Almost Always	Always
1	2	3	4	5	6

1. ____ When I accomplish something difficult I feel delighted or elated.
2. ____ When I feel happy it is a strong type of exuberance.
3. ____ I enjoy being with other people very much.
4. ____ I feel pretty bad when I tell a lie.
5. ____ When I solve a small personal problem, I feel euphoric.
6. ____ My emotions tend to be more intense than those of most people.
7. ____ My happy moods are so strong that I feel like I'm "in heaven."
8. ____ I get overly enthusiastic.
9. ____ If I complete a task I though was impossible, I am ecstatic.
10. ____ My heart races at the anticipation of some exciting event.
11. ____ Sad movies deeply touch me.
12. ____ When I'm happy it's a feeling of being untroubled and content rather than being zestful and aroused.
13. ____ When I talk in front of a group for the first time my voice gets shaky and my heart races.
14. ____ When something good happens, I am usually much more jubilant than others.
15. ____ My friends might say I'm emotional.
16. ____ The memories I like the most are those of times when I felt content and peaceful rather than zestful and enthusiastic.
17. ____ The sight of someone who is hurt badly affects me strongly.
18. ____ When I'm feeling well it's easy for me to go from being in a good mood to being really joyful.
19. ____ "Calm and cool" could easily describe me.
20. ____ When I'm happy I feel like I'm bursting with joy.
21. ____ Seeing a picture of some violent car accident in a newspaper makes me feel sick to my stomach.
22. ____ When I'm happy I feel very energetic.

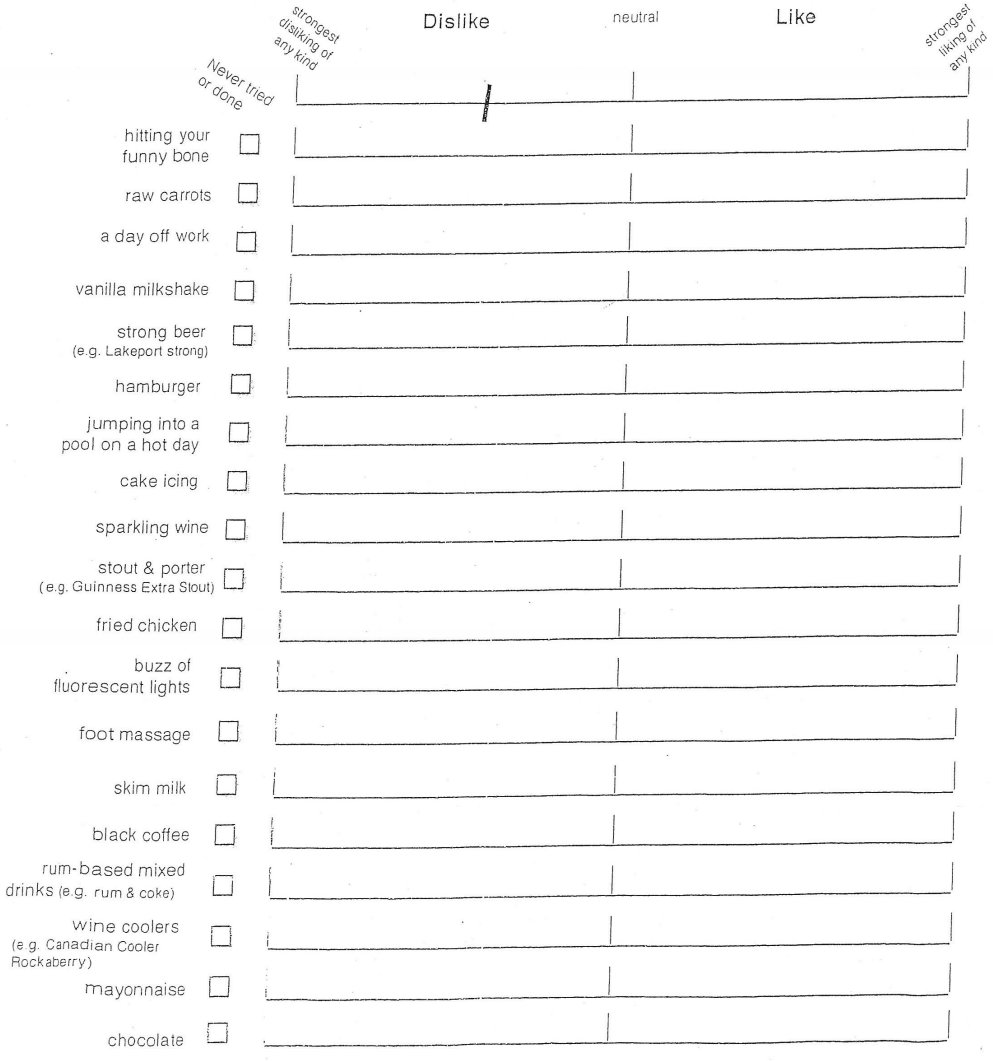
23. ____ When I receive an award I become overjoyed.
24. ____ When I succeed at something, my reaction is calm contentment.
25. ____ When I do something wrong I have strong feelings of shame and guilt.
26. ____ I can remain calm even on the most trying days.
27. ____ When things are going good I feel "on top of the world."
28. ____ When I get angry it's easy for me to still be rational and not overreact.
29. ____ When I know I have done something very well, I feel relaxed and content rather than excited and elated.
30. ____ When I do feel anxiety it is normally very strong.
31. ____ My negative moods are mild in intensity.
32. ____ When I am excited over something I want to share my feelings with everyone.
33. ____ When I feel happiness, it is a quiet type of contentment.
34. ____ My friends would probably say I'm a tense or "high-strung" person.
35. ____ When I'm happy I bubble over with energy.
36. ____ When I feel guilty, this emotion is quite strong.
37. ____ I would characterize my happy moods as closer to contentment than to joy.
38. ____ When someone compliments me, I get so happy I could "burst."
39. ____ When I am nervous I get shaky all over.
40. ____ When I am happy the feeling is more like contentment and inner calm than one of exhilaration and excitement.

Appendix XV: Food Liking Questionnaire

ID# _____ generalized Degree of Liking scale - v1.1

The ends of the scale represent the strongest liking or disliking of any kind.

Place a vertical line (not an X) anywhere on the line. For example, decide how much you like the sensation of walking out of a dark movie theater on a bright sunny day. As shown, my rating for this sensation is between neutral and strongest disliking of any kind.



ID# _____

generalized Degree of Liking scale - v1.1

The ends of the scale represent the strongest liking or disliking of any kind.

	Never tried or done	strongest disliking of any kind	Dislike	neutral	Like	strongest liking of any kind
burn of a spicy meal	☐					
cereal	☐					
cheddar cheese	☐					
red wine	☐					
french fries	☐					
walking barefoot on hot pavement	☐					
sausage	☐					
rice	☐					
lager beer (e.g. Heineken, Molson Canadian)	☐					
diet coke/pepsi	☐					
getting a paycheck	☐					
dry cider (e.g. Magners, Strongbow)	☐					
cotton candy	☐					
smell of fresh flowers	☐					
sweet white wine	☐					
wind on your face on a winter day	☐					
pizza	☐					
fresh strawberries	☐					
whisky-based mixed drinks (e.g. Rye and Ginger Ale)	☐					
steak	☐					
vodka-based mixed drinks (e.g. Mike's Hard Lemonade, Woody's)	☐					
cream	☐					

ID# _____

generalized Degree of Liking scale - v1.1

The ends of the scale represent the strongest liking or disliking of any kind.

	Never tried or done	Strongest disliking of any kind — Dislike — neutral — Like — strongest liking of any kind
ice cream	☐	
sound of a child laughing	☐	
cookies	☐	
Pancakes / Waffles	☐	
plain broccoli (cooked)	☐	
smell of used gym socks	☐	
scotch/whisky (straight/with ice)	☐	
apples	☐	
vodka (straight/with ice)	☐	
canteloupe	☐	
cinnamon rolls	☐	
ale (e.g. Molson Export, Newcastle Brown Ale)	☐	
donuts	☐	
regular coke/pepsi	☐	
dry white wine	☐	
white bread	☐	
bacon	☐	
India Pale Ale (e.g. Dogfish Head, Deuchars IPA)	☐	
hot dog	☐	
whole wheat bread	☐	
pastries	☐	
light beer (e.g., Coor's light)	☐	

ID# _____ generalized Degree of Liking scale - v1.1

The ends of the scale represent the strongest liking or disliking of any kind.

	Never tried or done	strongest dislking of any kind	Dislike	neutral	Like	strongest liking of any kind
wasabi	☐					
Brussels Sprouts	☐					
gin (straight/with ice)	☐					
blueberries	☐					
Chips	☐					
jalapeno peppers	☐					
rum (straight/with ice)	☐					
glare of headlights at night	☐					
pasta	☐					
energy drinks (e.g. Red Bull)	☐					
whole milk	☐					
tonic water	☐					
fortified wine (e.g. port, sherry)	☐					
green beans	☐					
warm fire on a cold day	☐					
plain broccoli (raw)	☐					
brandy/cognac (straight/with ice)	☐					
unsweetened grapefruit juice	☐					
Brownies	☐					
skim milk	☐					
liquers (e.g. Bailey's Irish Cream)	☐					
Salted popcorn	☐					

Thank you very much for completing this survey!

Appendix XVI: Food Behaviors Questionnaire

ID: _____

General Food Behaviours
Check the box next to each statement that best applies to you

	strongly agree			neither agree nor disagree			strongly disagree	can't answer
I am constantly sampling new and different foods/beverages	☐	☐	☐	☐	☐	☐	☐	☐
I don't trust new foods/beverages	☐	☐	☐	☐	☐	☐	☐	☐
If I don't know what is in a food, I won't try it	☐	☐	☐	☐	☐	☐	☐	☐
I like foods/beverages from different countries	☐	☐	☐	☐	☐	☐	☐	☐
Ethnic foods/beverages look too weird to eat/drink	☐	☐	☐	☐	☐	☐	☐	☐
At dinner parties, I will try a new food	☐	☐	☐	☐	☐	☐	☐	☐
I am afraid to eat/drink things I have never had before	☐	☐	☐	☐	☐	☐	☐	☐
I am very particular about the foods I will eat	☐	☐	☐	☐	☐	☐	☐	☐
I will eat almost anything	☐	☐	☐	☐	☐	☐	☐	☐
I like to try new ethnic restaurants	☐	☐	☐	☐	☐	☐	☐	☐

How often do you try unfamiliar foods?	Never	☐
	Rarely	☐
	Some of the time	☐
	Most of the time	☐
How often do you try unfamiliar alcoholic beverages?	Never	☐
	Rarely	☐
	Some of the time	☐
	Most of the time	☐